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Ozone and Other Photochemical Oxidants

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This report has been critically reviewed according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. Only after completion of the review process has it been released for publication.

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CHAPTER 8

TOXICOLOGY*

The toxicologic aspects of oxidant air pollution have most often been examined through laboratory studies of individual constituents of the complex mixture of air contaminants that characterizes the ambient air environment. Photochemical air pollution (with automobile exhaust as the primary emission source) can be chemically characterized as oxidizing in reaction, and the chemical species that contribute to this oxidizing property are varied. Among the strongest of the photochemically formed oxidants that are present in "oxidant smog" and are stable enough to be identified and measured is ozone. Because ozone is also known to be among the most toxic of gases, it is logical that most toxicologic research related to the potential health effects of photochemical air pollution has focused on it. It has become almost a reflex to think of ozone toxicity when "oxidant toxicity" is mentioned. Nitrogen dioxide is another toxic gas present in "oxidant smog," but, by most conventional indexes, it is less toxic than ozone, especially when compared in the same proportions as their concentrations in ambient air. Indeed, ozone and nitrogen dioxide share many common toxicologic properties. Nitrogen dioxide will be discussed in another monograph in this series, so its toxicology will not be reviewed here, except for specific comparisons. Other chemical species--e.g., the peroxyacylnitrates and hydroxyl free

*Most experimental toxicology studies of inhaled vapors and gases report concentrations as parts per million. That convention is used in this chapter. See Chapter 6 for relations of various measurement units.

radicals, which undoubtedly contribute to the oxidizing properties of the total mixture of oxidant air pollution—may also contribute to the total biologic activity of this mixture. However, there have been too few controlled toxicologic studies to permit an evaluation of their contribution to the toxicologic actions of photochemical oxidant mixtures. For example, the single toxicologic study on PAN that has been reported indicated that it is much less acutely toxic than ozone (Campbell et al., 1967)[27].

The review of the experimental toxicology of photochemical oxidants in this chapter is therefore primarily a review of the toxicology of ozone. The reader should bear in mind that what is said about ozone may not apply in every case to the broader term "photochemical oxidants", but, given the current state of knowledge, ozone toxicology is the best approximation of the toxicology of the class. With this in mind, it is of value to review briefly the experimental toxicology of complex mixtures of ambient or laboratory simulated photochemical smog.

Investigations of the potential biologic actions of a complex photochemical reaction mixture, produced by irradiating mixtures of air and auto exhaust under laboratory conditions that simulated real driving patterns and solar irradiation, were first conducted in the laboratories of the Division of Air Pollution, U.S. Department of Health, Education, and Welfare (DHEW)[105,106,109,147,150]. The effects of both nonirradiated and irradiated exhaust mixtures were studied. Clearly, irradiation of the air-exhaust mixture led to the formation of photochemical reaction products that were biologically more active. The changes in chemical qualities of the mixture

that accompanied the increased biologic activity were an increase in the concentration of total oxidant and an increase in the formation of irritant aldehydes. The relative proportions of the suspect biologically active chemical species varied with the total concentration of exhaust gases in the irradiated mixture and with the duration of irradiation.[147,150]

Single-inhalation exposure studies lasting a few hours demonstrated in laboratory animals that irradiation of exhaust mixtures led to greater effects on respiratory mechanics in guinea pigs, greater reduction in voluntary running activity of mice, and slightly greater carboxyhemoglobin formation in rats, compared with animals exposed to the same total concentration of exhaust gases that were not irradiated. The concentration of total oxidant in these experiments ranged between 0.33 and 0.80 ppm in the irradiated-exhaust mixtures; only a trace or no oxidant was detected in the unirradiated exhaust. The irritant aldehydes, formaldehyde and acrolein, were also present in higher concentrations in the irradiated atmospheres. The effects that were noted during the period of exposure (usually 4-6 h) were reversible within a few hours when the animals were returned to clean air. The effects in animals exposed to the irradiated-exhaust mixture are not necessarily uniquely characteristic of ozone, but most of them could have been produced by ozone.

The nature of changes in respiratory mechanics in guinea pigs exposed to irradiated exhaust varied somewhat according to the ratio of oxidant to aldehyde concentrations (formaldehyde and acrolein were measured), and this ratio in turn varied with the duration of irradiation of the air-exhaust mixture.[147] Thus, when the oxidant:aldehyde ratio was low, the

pattern of effects on guinea pig respiration resembled that produced by such upper airway irritants as formaldehyde and acrolein [6,149,152] and was characterized by increased pulmonary flow resistance, increased tidal volume, and decreased frequency of breathing. Increasing the ratio resulted in a shift in the pattern of respiration toward that produced by deep-lung irritants (e.g., ozone and nitrogen dioxide) [153] --namely, decreased tidal volume and increased frequency--although the increased flow resistance typical of the aldehyde effect persisted. This interactive effect of an oxidant-aldehyde mixture could be reproduced by a simple mixture of ozone and acrolein.[147] Concentration-response relationships of these effects and other actions in laboratory animals exposed to mixed or single components of oxidant air pollution are summarized in Table 8-1.

From the data in Table 8-1, the conclusion could be drawn that all the functional effects observed in short-duration experiments with laboratory-produced photochemical smog mixtures could have been due to ozone alone if one considers total oxidant concentration of the mixture equivalent to ozone concentration. A possible exception is the increase in respiratory flow resistance in guinea pigs, which is more characteristic of and probably due, at least in part, to the irritant aldehydes in the exhaust mixtures. The increase in respiratory frequency in guinea pigs is most probably due to the oxidant (or ozone) content of the mixture. Comparison of the concentrations for equal effectiveness in decreasing the spontaneous running activity in mice also suggests that this action of the mixture may be largely due to ozone; and the oxidant content of irradiated auto exhaust appears to explain adequately the increase in

Table 8-1

Effects of Photochemical Oxidant Air Pollution Mixtures (in Irradiated Auto Exhaust) and Some Individual Constituents in Single Short (6-h) Exposures[a]

Effect	Air Pollutant	Concentration, ppm	Reference
50% increase in respiratory flow resistance in guinea pigs	Formaldehyde	3.9	152
	Acrolein	0.6	149
	Ozone	1.1	153
	Mixture:		
	Formaldehyde	1.93	147
	Acrolein	0.09	147
	Oxidant	0.91	147
	Nitrogen dioxide	2.00	147
	Carbon dioxide	250	147
50% increase in respiratory frequency in guinea pigs	Formaldehyde	(decreased respiratory frequency)	152
	Acrolein	(decreased respiratory frequency)	149
	Nitrogen dioxide	6.5	153
	Ozone	0.68	153
	Mixture:		
	Formaldehyde	1.32	147
	Acrolein	0.06	147
	Nitrogen dioxide	0.79	147
	Oxidant	0.95	147
	Carbon monoxide	150	147
50% decrease in voluntary running activity in mice	Acrolein	0.4	147
	Nitrogen dioxide	16	153
	Ozone	0.3	147
	Carbon monoxide	250	147
	Mixture:		
	Formaldehyde	1.11-1.39	147,150
	Acrolein	0.09-0.10	147,150
	Nitrogen dioxide	1.64-2.95	147,150
	Oxidant	0.45-0.82	147,150
	Carbon monoxide	85-95	147,150

Table 8-1 (cont.)

Effects of Photochemical Oxidant Air Pollution Mixtures
(in Irradiated Auto Exhuast) and Some Individual Constituents
in Single Short (6-h) Exposures[a]

Effect	Air Pollutant	Concentration, ppm	Reference
Significant increase in susceptibility to induced respiratory infection in mice	Ozone	0.08	38
	Nitrogen dioxide	3.5	54
	Mixture:		
	Oxidant	0.15	38
	Carbon monoxide	25	38

[a] Data shown are summarized and adapted from dose-response curves, typical concentration values, etc., given in references cited. The data should therefore be considered approximations and used only for comparison.

susceptibility to respiratory infection in mice exposed to the mixture. These conclusions must, of course, be qualified by the possibility that other chemical species, such as hydroxyl free radicals, that were not measured or reported might also have produced the same effects and led to the same conclusions. Nevertheless, it is reasonable to conclude that many of the functional effects produced by short-duration exposures to complex photochemical oxidant mixtures are due to ozone.

Hueter et al.[109] exposed animals to irradiated automobile exhaust (simulated photochemical smog) for periods up to 23 months. The concentrations were cycled, to simulate daily peak and trough pollution concentrations in urban centers. Peak daily concentrations of carbon monoxide were 20, 50, 60, and 100 ppm in four sets of exposure chambers. There was considerable loss of ozone and nitrogen dioxide on chamber walls, cages, and animal fur, so the concentrations of chemically reactive gases to which the animals were exposed probably ranged from about 0.04 to 0.2 ppm for ozone and about 0.15 to 0.5 ppm for nitrogen dioxide. Pulmonary flow resistances, tidal volumes, and respiratory frequencies of guinea pigs were measured (while the animals were breathing clean air for brief test periods) at 16-week intervals during the experiments and were not affected by the chronic exhaust exposures. Exhaust-exposed mice showed a decrease in running activity for the first few weeks of exposure, but then recovered to control activity. A decrease in mouse fertility rate and a decrease in infant survival rate occurred in the exhaust chambers, and there was an increase in the rate of spontaneous pulmonary infection in exhaust-exposed animals. There were no significant effects of exhaust

exposures on mortality, histopathology, growth rate, or hematologic indexes. Interpretation of these studies is difficult, because of surface loss of contaminants in the chambers. The decrease in mouse fertility (duplicated in a second experiment [125]), the decrease in mouse physical activity, and the increase in susceptibility to infections suggest that some functional measurements were more sensitive for detecting effects than conventional histologic, hematologic, or growth measurements. It is doubtful, however, whether any of the observed changes could be considered chronic effects, i.e., irreversible or progressive in the absence of continued exposure.

Wayne and Chambers [216] reviewed the findings of studies on experimental animals exposed throughout their lifetimes to ambient Los Angeles atmosphere. The control animals were kept in rooms that were ventilated with special filters that removed most of the ambient air pollutants. No clear evidence of chronic injury from the ambient air pollution was observed. There was some suggestion from pulmonary function tests, electron-microscopic examinations, and pulmonary adenoma incidence that aged animals were adversely affected by ambient smog; and some reversible changes in pulmonary function of guinea pigs were noted during peak periods of air pollution. Increased 17-ketosteroid excretions suggested that breathing polluted ambient air was stressful for guinea pigs. Not only were the reported effects marginal, but some of them may have been due to variations in temperature and humidity. Although the information obtained is of little value in the quantitative assessment of the health hazards of air pollution, some subjects for possible future consideration were revealed:

the effects of air pollutants on aged animals in which physiologic repair processes are less active and the effects of other stressful stimuli on the response to air pollutants.

Emik and Plata[55] reported on studies of the effect of breathing ambient oxidant-polluted air in Riverside, California, on spontaneous running activity of mice, as recorded over a 1-year period. The controls were mice that were exposed to highly filtered air. Monthly peak oxidant concentrations in the unfiltered ambient air were 0.05-0.24 ppm; peak nitrogen dioxide concentrations, 0.04-0.07 ppm; and peak total hydrocarbon concentrations, 2.7-4.4 ppm. The daily activity of mice breathing filtered air was consistently higher than that of those breathing ambient air, and the differences between the two groups were greatest during the months when peak oxidant concentrations were highest. Other environmental variables, especially temperature, may also have contributed to the magnitude of differences between the groups.

Nakajima *et al.*[160] studied histopathologic changes in the lungs of mice that were exposed to irradiated auto exhaust and oxidant-fortified exhaust-gas mixtures for 2-3 h/day on 5 days/week for a month. Histopathologic changes resembling tracheitis and bronchial pneumonia were observed in mice exposed to atmospheres containing oxidant at 0.1-0.5 ppm, but not in those exposed to atmospheres containing 0.1-0.15 ppm. The changes were minimal in the latter group; the main finding reported was irregular arrangement of the epithelial cells of the relatively thick bronchioles.

As will be shown in greater detail, ozone alone can produce many of the effects of complex mixtures of photochemical oxidant air pollution

that have been reported from studies on experimental animals. Studies of the response of animals to these complex mixtures have the advantage that they should detect the gamut of potential toxicity of photochemical oxidant smog. However, the study of complex mixtures has limited value for mechanism studies, the most useful type of research for laboratory animal experimentation. The mere demonstration of similarity in quality of response to complex mixtures and selected individual constituents does not ensure that the constituents studied are the only or even the primary biologically active ones. At the same time, lack of similarity, both qualitative and quantitative, between effects of a mixture and effects of individual constituents may reflect interactive effects, both synergistic and antagonistic. The prime disadvantage of attempting to study the complex mixture of photochemical smog—either ambient smog or a realistic laboratory simulation—is the difficulty of obtaining reproducible, controlled atmospheres.

EFFECTS IN THE LUNGS

Disposition and Uptake

There is little information on the exact disposition and sites of uptake of ozone. However, because it is relatively insoluble in water, it can reach the periphery of the lung and hence has the opportunity to exert damage to both the central airways and terminal lung units. Ozone is highly reactive, and, given the concentrations found in ambient air, it is highly unlikely to be exhaled as ozone under conditions of normal breathing.

Frank and colleagues [70,222] have attempted to define the amount and site of uptake of ozone in the lung, by two methods: direct measurement

of the drop in concentration of the gas across the upper airway (nose and pharynx) or the remainder of the airways, and predictions based on a computer model. In their animal experiments, they used anesthetized, paralyzed, mechanically ventilated beagles. The upper airways were surgically isolated, and ozone at two concentrations (0.7-0.85 and 0.2-0.4 ppm) was administered by nose or mouth. Flow rates were either high (35-45 liters/min) or low (3.5-6.5 liters/min). Each exposure lasted 20-30 min; but, because of the slow response of the mast ozone meter, measurements of respiratory uptake were useful only during the last 4-5 min of exposure. The following observations were made: nasal uptake exceeded oral uptake at both high and low flow rates ($p<0.01$); uptake of ozone, both nasally and orally, was inversely related to flow rate ($p<0.01$), except when the gas was directed through the mouth at the high flow rate; and a decrease or increase in nasal resistance induced by drugs did not significantly change the uptake. The same investigators have developed a computer model for predicting uptake of ozone, with some assumptions related to solubility coefficient, an idealized version of the morphometry of the lungs[217] and a fixed set of ventilatory parameters. Computer analysis has shown that the dosage of ozone is greater to the peripheral airways than to the alveolar structures.[70]

Effects of Short-Term Exposure to Ozone

Morphologic Effects. Gross autopsy findings of pulmonary edema and hemorrage after acute exposure to ozone have been known for some time. Several studies have shown that edema and an acute inflammatory response occur in several species after brief exposures to ozone. The minimal

concentration of ozone that causes the responses that have been demonstrated depends very much on the methods used for the detection of edema.

Scheel et al.[191] provided histopathologic evidence of injury caused by a single acute exposure to ozone at 1.0 or 3.2 ppm for 4 h in mice and by repeated intermittent exposures (8-45 ppm for 1 h) in rabbits. No gross pulmonary edema was observed in mice killed immediately after exposure to 1 ppm, but moderately engorged blood vessels and capillaries containing an excess of leukocytes were found. Mice killed 20 h after exposure showed mild edema and migration of the leukocytes into the alveolar spaces. Inhalation of 3.2 ppm produced grossly visible edema during or shortly after exposure. The perivascular lymphatic vessels were distended and filled with edematous precipitate. Hyperemia (excess blood), mobilization of leukocytes, and various degrees of extravasation of red cells accompanied the edema. Damage to the respiratory tract consisted of loss of epithelium from the bronchioles; sheets of desquamated epithelial cells were seen in the lumen. The technique for measuring the gross edema produced (wet and dry lung weights) that was used in earlier studies was not sufficiently sensitive to detect a mild edematous reaction. More refined methods, based on the recovery of radiolabeled blood albumin in pulmonary lavage fluid, indicate that the ozone-exposure threshold for edema formation in rats is 0.25-0.5 ppm for 6 h.[5]

The use of sophisticated techniques of light and electron microscopy have enabled several investigators to describe in detail the nature of the inflammatory response. Boatman et al.[16] observed a desquamation of the ciliated epithelium throughout the airways of cats exposed to ozone

at 0.25, 0.5, and 1.0 ppm for 4-6 h. The intensity of the response appeared to be dose-related. Ultrastructural changes in the airways consisted of cytoplasmic vacuolization of ciliated cells and condensed mitochondria with abnormal cristae configuration. The latter occurred most often in the medium-sized airways, 0.8-1.2 mm in diameter. Alveolar ultrastructural changes included swelling and denudation of the cytoplasm of Type I cells, swelling or breakage of capillary endothelium, and lysis of red blood cells. Type II pneumocytes appeared normal. There was a considerable variation in the degree of response, both within a single lung and from animal to animal.

Stephens et al.[197] have shown that, in rats, the degenerative changes in Type I alveolar cells occur after exposure to ozone at concentrations as low as 0.2 ppm for 3 h and that the cells are replaced by Type II alveolar cells beginning a day after the exposure. By electron microscopy, Bils[11] noted that the swelling of the epithelial alveolar lining cells of mice occurred after exposure to ozone at 0.6-1.3 ppm for 4 h. These changes were not accompanied by fluid accumulation in the alveolar space. There was also a focal swelling of the endothelial cells and an occasional break in the basement membrane.

Electron microscopy has also shown that, in rats exposed to ozone at 3 ppm for 4 h and in mice exposed to 4 ppm for 3 h, acute inflammatory bronchiolar lesions occur.[174,220] These concentrations were high enough to produce alveolar edema, and the observed changes were similar to those found in earlier studies that used standard histologic techniques.

Effects on Pulmonary Function. Changes in pulmonary function have been observed in a variety of species after short-term exposure to ozone, including alterations in the elastic behavior of the lungs, increased resistance to flow, and decreased carbon monoxide diffusion capacity.

Scheel et al.[191] exposed 75 rats to ozone at 2 ppm for 3 h and measured pulmonary function immediately after removal of the animals from the exposure chamber. Decreases in minute ventilation, tidal volume, and oxygen uptake occurred immediately after exposure and reached minimal recorded values after 8 h. At 20 h after exposure, all measurements had returned to normal. Pulmonary edema may have been responsible for the observations reported.

Murphy et al.[153] exposed guinea pigs to ozone at 0.34-1.35 ppm for 2 h. Respiratory rates increased and tidal volumes decreased during exposure to all concentrations. The maximal changes were significantly different ($p < 0.05$) from preexposure control values for every concentration used. Once a maximal response was reached, the effects tended to remain constant for the remainder of the test period. Respiratory rates and tidal volumes tended to return to preexposure control values when the animals were returned to clean air. Total respiratory flow resistances were not significantly altered during inhalation of ozone at 0.34 and 0.68 ppm, but increased by 47% during exposure to 1.08 ppm.

Easton and Murphy[52] observed up to a threefold increase in flow resistance within an hour in guinea pigs during exposure to ozone at 5-7 ppm. There was a 50% increase in frequency of breathing and a small increase in tidal volume. Lung compliance decreased by 50%. After

exposure, all measurements returned to preexposure values within 3 h. These responses did not result in an increase in the work of breathing, in contrast with the response of guinea pigs to other irritants (formaldehyde and sulfur dioxide), in which the observed changes in pulmonary function resulted in an increase in the work of breathing.[7]

Recently, Watanabe et al.[215] observed changes in respiratory mechanics and single-breath diffusing capacity in cats exposed to ozone at 0.26-1.0 ppm for an average of 4-6 h. The animals were anesthetized, paralyzed, and mechanically ventilated through a tracheal cannula. Increased pulmonary flow resistance was the most sensitive index of ozone exposure in this species and occurred in almost all the animals exposed to 1.0 and 0.5 ppm and in two cats exposed to 0.25 ppm. Although the magnitude of the change was dose-related, it appeared that the concentration of ozone was more critical than the duration of the exposure. The mechanism of the response to ozone at 1.0 ppm appeared to differ from that to the lower concentrations in that there was a pulsatile increase in resistance that was superimposed on a more progressive change. The underlying increase in resistance was approximately exponential with respect to time. Changes in dynamic compliance and capacity for diffusing carbon monoxide were less frequent and less marked than the change in resistance. Vital capacity did not change. The shape of the volume-pressure curve during deflation did not change, and that suggests that alveolar stability was not affected. In cats, therefore, exposure to ozone affects airway caliber at concentrations lower than those which affect transfer of carbon monoxide across the alveolar-capillary membrane

or alveolar surface forces in the absence of pulmonary edema. Unlike the increase in pulmonary flow resistance that results from exposure to sulfur dioxide, the response to ozone is not mediated entirely via reflex bronchoconstriction, inasmuch as it was only partially reversed by atropine in these experiments. About half the response persisted after the administration of the drug. Thus, the small airways are implicated in the persistent increases in pulmonary flow resistance in response to ozone. This implication is further supported by the following observations: vagal innervation ends short of the peripheral airways, ozone can penetrate to the periphery of the lung, and postmortem studies show that inflammatory changes occur in the peripheral airways.

Unilateral exposure of the lung may be achieved in rabbits, because they have an intact mediastinum, which permits the unexposed lung to serve as a control for the exposed lung. The control lung may be collapsed, and ozone inhaled in a normal fashion by the noncollapsed lung; or, by selective catheterization, one lung can be made to breathe ozone through an airtight system while the other breathes ambient air. Rabbits so exposed to ozone at 12 ppm had increases in respiratory rate and minute volume and bilateral decreases in tidal volume and dynamic compliance.[4] Pulmonary function measurements were not significantly different in the ozone-exposed lung until edema developed, as determined by an increase in the wet weight of that lung. After edema formation, tidal and minute volumes, inspiratory and expiratory flow rates, and dynamic compliance decreased and pulmonary flow resistance increased in the lung exposed to ozone, relative to the unexposed lung.

Studies have been performed on the effects of ozone exposure on excised dog lungs.[69] This preparation offers the advantages of providing information on changes in pulmonary function in the absence of intra-alveolar edema and vagal innervation. High concentrations of ozone were used (6.8-10.3 ppm for 2.5 h and 5-10 ppm for 3 h). The changes in static-deflation volume-pressure relationships were barely detectable, whether measured in air or saline. The data suggest that exposure to ozone does not produce significant depletion or degradation of either surface-active material or tissue elastic elements in the absence of edema. Thus, it seems that the changes in elastic behavior, as determined by changes in dynamic lung compliance, are secondary to the formation of edema, unevenly distributed changes in flow resistance or compliance, and a change in tidal volume or end-expiratory lung volume.

There is evidence that ozone has a delayed effect on elastic behavior. Excised lungs of rabbits that were unilaterally exposed to ozone at 1.0 ppm for 3 h and then allowed to breathe ambient air for up to 2 weeks showed a depression in the volume-pressure curves. This change was present during most of the postexposure test period. The mechanism underlying this response was not determined.[70]

Frank et al.[71,72] exposed the right lung of rabbits to ozone at 2.2-12.1 ppm for 3 h, the left lung having been collapsed before exposure. When edema occurred in the right lung, changes in surfactant behavior (as determined by in vitro measurements of the surface tension of washings from the left lung) were observed in some animals. No such changes were observed in the absence of edema in the right lung. These results suggest

not only that ozone can induce chemical changes in exposed lungs, but that the products of such changes can cause deleterious effects in non-exposed lungs.

Effects of Prolonged Exposures to Ozone

According to Stokinger,[199] at least three effects of long-term exposure to ozone have been recognized: effects on morphology and function of the lung, lung-tumor acceleration, and aging. An additional effect, the development of tolerance after exposure to low concentrations of ozone, may also be related to chronic toxicity.

Stokinger et al.[204] reported that chronic bronchitis, bronchiolitis, and emphysematous and fibrotic changes in the lung tissues occur in mice, hamsters, and guinea pigs exposed daily to ozone at a concentration slightly above 1 ppm. These irreversible changes also develop in animals that have developed tolerance to acute inflammatory effects.

Freeman et al.[75] used tissue fixation and electron microscopy and reported on the morphologic changes that occurred in the lungs of dogs exposed to ozone at 1-3 ppm for 5-24 h/day for up to 18 months. In general, the effects reported tended to increase in severity with the concentration used, rather than with the duration of the exposure. There was no evidence of pulmonary edema. The most noticeable change was a thickening of the bronchioles and respiratory bronchioles. This change was barely noticeable at the lowest concentrations used, but at the highest concentrations it was accompanied by infiltration of lymphocytes, plasma cells, and fibroblasts that formed peribronchiolar collars, thus reducing the caliber of the small airways. Bronchiolar changes included an increase in the proportion of mucus-forming cells and squamous metaplasia

of columnar and cuboidal cells. In addition, there was an increase in the average number of macrophages per field along the peripheral airways.

In developing rats exposed to ozone at 0.54 or 0.88 ppm for up to 3 weeks, morphologic changes were seen in the respiratory bronchioles and distal portions of the terminal bronchiolar epithelium, the entire alveolar duct, and the associated alveoli. These changes started to occur after 24 h of exposure; but, after several days of exposure, the hypertrophic bronchiolar epithelium tended to recede and was replaced by cuboidal or squamous cells. The lungs of growing rats exposed to ozone at 0.9 ppm were approximately 38% heavier than those of normal animals of the same age; within 3 weeks, 50% had died with grossly inflated lungs.[74]

Mice exposed to ozone at 2.5 ppm for 120 days showed progressive metaplasia along the tracheal bronchial tree. Return of these animals to clear air resulted in a reversal of these changes after a further 120 days.[170]

Bartlett et al.[10] exposed 194 young rats (3-4 weeks old) continuously to ozone at 0.2 ppm for 28-32 days and observed that there was no effect on respiratory frequency, weight gain, tail-length increase, and external appearance in the ozone-exposed group and that, although both ozone-exposed and control groups looked healthy, 12 ozone-exposed and 11 control animals had pneumonitis at the end of the exposure period. The results with the latter animals were discarded in the later data analysis. The lung volumes of the ozone-exposed group were 16% greater than those of the control group. Air or saline static volume-pressure deflation curves at 95% or 100% of total lung capacity showed that transpulmonary pressure

was significantly lower in the ozone-exposed group, thus confirming that exposed lungs were over distended at high transpulmonary pressures. The fact that this occurred in both air- and saline-filled lungs suggested a small change in tissue elasticity, rather than abnormal surface tension. There were no apparent morphologic differences between the two groups detected by conventional staining techniques, but more sensitive electron-microscopic techniques might have revealed some differences.

Acceleration of lung tumorigenesis (adenoma) in a lung-tumor-susceptible strain of mice occurred after daily exposures to ozone at about 1 ppm. At 15 months, an incidence of 85% was seen in the ozone-exposed animals, compared with 38% in the controls; the average number of tumors per mouse was 1.9, compared with 1.5 in the controls.[199]

There is some suggestive evidence that exposure to ozone accelerates the aging process(es). Bjorksten[12] and Bjorksten and Andrews[13] have presented evidence that aging is due to irreversible cross-linking between macromolecules, principally proteins and nucleic acids. Aldehydes were included in the list of cross-linking agents, and these can be produced in the lung[26] as a result of ozone exposure.

Tolerance

One feature of the response to oxidants (in particular, ozone) that has stimulated considerable interest is the apparent development of tolerance to the acute effects of short-term exposure to these agents in laboratory animals.[59] Fairchild reviewed possible mechanisms of this phenomenon. Tolerance has been defined as the increased capacity of an organism that has been preexposed to oxidant to resist the effects of

later exposures to ordinarily lethal (or otherwise injurious) doses of the same agent or of different agents (cross-tolerance) with similar toxicologic properties.

Development of tolerance to the acute toxicity of ozone in experimental animals has been demonstrated after a single, brief (1 h or less) exposure to low concentrations (0.3-3 ppm). Rats can develop a tolerance that lasts for a month or longer. In mice, a tolerance lasting up to 14 weeks has been observed. Ozone is not the only lung edematogenic agent that can produce tolerance. This phenomenon is also observed with nitrogen dioxide, phosgene, and phenylthiourea. The tolerance evoked by one agent can provide cross-protection against other irritants. For example, a single exposure of rats or mice to ozone (0.5-5 ppm for 1-5 h) will induce protection against the acute pulmonary effects of nitrogen dioxide, hydrogen peroxide, ketene, phosgene, hydrogen sulfide, and nitrosyl chloride.[59] The development of pulmonary edema is the toxic effect of all these compounds. Although pretreatment by injection of phenylthiourate will provide tolerance to inhalation of an ordinarily lethal dose of ozone, the reverse is not true. Inhalation of ozone will not provide cross-tolerance to the lung edema produced by injection of thiourate.

Studies by Henschler *et al.*[104] on the development of tolerance to nitrogen dioxide in mice provided some insight into the significance of this phenomenon. They found that mice that had been exposed only once to nitrogen dioxide were almost totally protected from effects of later exposures to high, usually fatal concentrations, whereas those which were

repeatedly exposed were only partially protected. It was concluded that the first exposure protected from the effects of the following exposures, thus inhibiting increased tolerance buildup. It is significant that, in another experiment, mice tolerant to nitrogen dioxide were exposed to a concentration high enough to cause lethality, but none of the mice died with pulmonary edema. The predominant gross finding was massive hemorrhage.

In animals made tolerant to ozone and later exposed to ordinarily injurious concentrations, the usual increase in lung water and other pathologic changes associated with edema were either reduced or absent, and the usual ozone-induced changes in activity of serum alkaline phosphatase and adrenal succinic dehydrogenase were absent or slight. In addition, there was significantly less oxidation of lung reduced glutathione in the tolerant animals.[59] It should be pointed out that there may be species differences in tolerance development. Quilligan et al.[182] used several different conditions of exposure and challenge and were unable to demonstrate tolerance to ozone in baby chicks; and it is not known whether humans develop tolerance to ozone.

Tolerance appears to be a local phenomenon, in that experiments with unilateral exposure showed that a lung is not protected from pulmonary edema unless it has been exposed itself. Thus, no tolerance was produced in the contralateral, unexposed lung; this suggested that there is no evidence of a circulating humoral agent that confers tolerance.[77] The same investigators also attempted to show whether induction of tolerance affected the cytotoxic effects of ozone on alveolar macrophages (discussed

in the next section). They used unilateral exposure and lavage and observed no differences in the cytotoxicity of ozone between tolerant and nontolerant lungs. A twofold increase in the number of polymorphonuclear neutrophilic leukocytes occurred in the tolerant lung. Alveolar-macrophage enzyme activities were equally depressed in both tolerant and nontolerant lungs. This study suggested, therefore, that tolerance does not protect the antibacterial defense mechanisms of the lung from the effects of ozone. In addition, Murphy et al.[153] found that exposure to ozone, which produced tolerance in guinea pigs to the edema produced by later exposure to high concentrations, failed to produce tolerance to the effects of low concentrations of ozone on respiratory frequency and tidal volume.

It appears that the development of tolerance is a useful tool to determine the mechanism of ozone-induced pulmonary edema. However, in the light of the findings that repeated intermittent exposures of mice to nitrogen dioxide produced less protection from the effects of a lethal dose than a single preexposure and the failure to produce tolerance to the effects of ozone on antibacterial defense mechanisms or on the respiratory patterns of guinea pigs, it is unlikely that tolerance development provides significant protection in human populations continuously exposed to low concentrations of oxidants.

Defense Mechanisms

It has been observed that animals challenged with aerosols of infectious organisms suffer a higher incidence of infection if they have been previously exposed to ozone, irradiated auto exhaust, or other common pollutants. The suggested explanation for this is that the various

polutants inhibit, inactivate, or otherwise impair two distinct functions: mucociliary streaming, the action of cilia in the nasal and upper respiratory passages that clears particles and thus prevents them from entering the lungs; and phagocytosis by alveolar macrophages.

Miller and Ehrlich[139] determined the effect of exposure to ozone on the susceptibility of mice and hamsters to respiratory infection caused by inhalation of Klebsiella pneumoniae aerosol. The ozone exposures used were 1.3-4.4 ppm for 3 h and 0.84 ppm for 4 h per day, 5 days/week for 2 weeks. The observation period for this experiment was 2 weeks. Mortality and survival time were measured. The mortality due to K. pneumoniae infection was significantly greater ($p < 0.05$) for every exposure regimen in which animals were exposed to ozone than in their paired controls. No deaths were caused by ozone exposure alone. Autopsy of the animals exposed to K. pneumoniae that died within the 14-day holding period showed the infectious organism in the lungs and heart. K. pneumoniae was absent in animals that survived the 14-day holding period. It was concluded that exposure to ozone significantly reduced the resistance of mice and hamsters to later respiratory infection due to K. pneumoniae. Statistical evaluation of the data indicated higher mortality, shorter survival time, and a lower LD_{50} (for K. pneumoniae) in animals exposed to ozone than in controls.

In a similar series of experiments conducted by Purvis et al.,[181] mice were exposed to ozone at 3.8-4.1 ppm for 3 h, 1-27 h before and 3-27 h after challenge with K. pneumoniae aerosol. Within 19 h after exposure to ozone, the resistance of mice to respiratory infection

initiated by challenge with the aerosol was significantly reduced. The same effect was observed in infected animals exposed to ozone up to 27 h after challenge with the aerosol.

Coffin and Blommer[36] have shown that mice exhibit increased mortality from Streptococcus aerosol after exposure to ozone at 0.08 ppm for 3 h. This concentration of ozone also produced a decrease in the rate of kill of bacteria deposited in the lungs and hence an increase in their later multiplication.[35] This effect was noted 4 days after exposure, but not 4 h after exposure.

Goldstein et al.[95] studied the effect of ozone on the in vivo rate of bacterial killing in the mouse lung. They used male Swiss mice infected with aerosols of Staphylococcus aureus labeled with phosphorus-32. The animals were sacrificed immediately or after exposure to ozone at 0.62-4.25 ppm for 4 h, and the radioisotope concentration in the lungs was determined. Increased numbers of bacteria were consistently cultured from the lungs of animals exposed to ozone, compared with controls, and the magnitude of the effect was dose-related up to 2.58 ppm. Some mice exposed to 2.2 ppm or greater yielded more staphylococci than were inhaled initially; thus, intrapulmonary bacterial multiplication had occurred. The difference between control and infected animals was significant ($p < 0.05$) at 1.10 ppm, when individual aerosol experiments were compared. When the data from all the experiments were pooled, there were significant differences in bacterial activity between control and treated mice for each ozone exposure ($p < 0.05$). Inasmuch as the ozone-exposed mice were able to remove the same number of bacteria as control mice via mucociliary

streaming, the investigators concluded that the inhibition of bactericidal activity resulted from the toxic effect of ozone on the alveolar macrophages.

Alpert and Lewis[3] used unilateral exposure to study the effect of ozone on the defense mechanisms of the lungs. Rabbits were exposed unilaterally to ozone at 0.5, 0.75, and 3 ppm for 3 h. The alveolar macrophages were harvested by pulmonary lavage. Although there was a statistically significant reduction in the viability of the cells from the ozone-exposed lung, compared with the air-exposed lung, the differences were small. There was also a dose-related inhibition of the intracellular hydrolytic enzymes acid phosphatase, lysosyme, and β-glucuronidase. The fraction of total cells present as polymorphonuclear neutrophilic leukocytes in the lavage fluid from the exposed lung increased in a dose-related fashion from approximately 2% at 0 ppm to approximately 28% at 3 ppm. Associated with the increase in the fraction of polymorphonuclear neutrophilic leukocytes was a reciprocal decrease in the fraction of alveolar macrophages, although the total number of macrophages per gram (dry wt) remained unchanged. The authors suggested that the depression of the intracellular hydrolytic enzymatic activity by ozone may contribute to the ozone-caused impairment of lung defense mechanisms.

Interactions with Bronchoactive Agents

The lung is relatively rich in histamine-containing mast cells, so it is not surprising that a number of investigators have assessed the role of histamine in the pulmonary toxicity observed after ozone exposure.

Among the many effects of histamine are edematogenic alterations in vascular endothelium and bronchoconstriction. The latter may be

particularly pertinent, in view of its being a central manifestation of asthma attacks and of the reported association of asthma attacks with photochemical air pollution. Bronchoconstriction has also been observed in animals and man experimentally exposed to ozone or photochemical air pollution.

Dixon and Mountain[49] reported that exposure of mice to ozone at 1 ppm for 5 h resulted in a depletion of lung histamine content to as low as 75% of the control value 5 days later. They also observed that pretreatment with promethazine, an antihistaminic agent, resulted in a decrease in the amount of pulmonary edema produced by a sublethal dose of ozone. However, promethazine, in addition to being a potent antihistaminic agent, is a phenothiozine derivative and thus might act as a free-radical-trapping or membrane-stabilizing agent.

Other investigators have been unable to demonstrate lung histamine depletion in rats exposed to ozone at 4 ppm for 4 h[41] or guinea pigs exposed to ozone at 1-5 ppm for 3 h.[52] In the latter study, it was observed that exposure to ozone at 5 ppm for 2 h followed by challenge with histamine at 0.9-1.4 mg/kg (injected 1.5-2.0 h after the end of exposure) resulted in increased mortality, compared with that in an air-exposed control group. The increased susceptibility to histamine was detectable for 120 h after the end of exposure to ozone at as low as 0.8 ppm. This concentration is about one-twentieth of that required to produce death from pulmonary edema due to ozone alone. It was found that the increased susceptibility occurred only when exposure to ozone took place before challenge with histamine. There was no increase in susceptibility in guinea pigs that received histamine before exposure.

Potentiation of the action of acetylcholine, another bronchoconstrictive agent implicated as a mediator in asthma, has also been reported in guinea pigs exposed to ozone at at least 2 ppm for 30 min before inhalation of acetylcholine;[128] the effect was observed intermittently for up to 23 h after ozone exposure.

The reported potentiation by ozone of the membrane damage produced by the indirect pathway of complement[88] might also play a role if it occurs *in vivo*, inasmuch as this complement pathway apparently mediates the allergin-reagin-induced release of histamine.[127] In view of the fact that asthma attacks usually occur at night, whereas ozone peaks in ambient air occur late in the morning, it might be more reasonable to ascribe the epidemiologic association of photochemical oxidants with asthma (if such exists—see Chapter 10) to an ozone-induced alteration of the respiratory tract, either potentiating an otherwise subclinical effect of an allergen or increasing the reactivity of smooth muscle to bronchoactive compounds.

The vasoactive compound serotonin has also been suggested to be responsible for ozone-induced pulmonary edema by Skillen *et al.*,[196] who noted a decrease in lung serotonin after ozone exposure. Prostaglandins may also be involved in ozone toxicity, in view of a report that aspirin, a prostaglandin inhibitor, protects from ozone toxicity.[49] Prostaglandins are released from injured lungs, possibly as a result of membrane damage.[173] Recently, Tan *et al.*[206] demonstrated that injection of hydroperoxides, which results in lung damage similar to that caused by injected ozonides,[39] appeared to interfere with lung prostaglandin synthesis.

In a study of the mechanism whereby *Bordetella pertussis* vaccine increased acute ozone toxicity in rate,[60,209] Thompson[208] ascribed the effects to β-adrenergic blockade, and not to an immune-mediated response. It was further noted that both atropine and reserpine reduced mortality,

which suggested that the acute lethal effects of ozone were due to shock and circulatory collapse, rather than pulmonary edema.

The release of bronchoactive compounds may result from immune reactions. A possible role for the immune system in chronic ozone toxicity was suggested by Scheel et al.[191] and by Stokinger and Scheel,[203] who hypothesized that ozone altered lung protein, making it immunogenic to the host. Presumably, the resulting antibody would cross-react with normal lung tissue and produce damage. Although some indirect evidence to support this hypothesis was presented,[25] it has been questioned. In addition, the suggestion that neonatal thymectomy altered ozone toxicity[98] has not been confirmed.[209]

BIOCHEMICAL ACTIONS AND MECHANISMS

Molecular Reactions

Free-Radical Effects of Ozone: The idea that ozone toxicity is expressed through the formation of reactive free-radical intermediates was originally derived from studies that noted the similarity of the effects of ozone to those of radiation. Earlier studies included the observation of a similar and generally additive effect of ozone and x irradiation in producing chromosomal aberrations in Vicia fava seeds;[64] radiation-like effects on the deoxygenation of hemoglobin in the occluded digits of humans breathing ozone at 1 ppm for 10 min;[20] the protective effects of thiol radioprotective agents in ozone toxicity;[62] the ability of ozone to produce mutations and chromosomal damage; and an increase in the radiation-induced sphering of human and animal red cells in vitro after ozone inhalation.[21] These studies and the idea of free-radical effects of ozone have been reviewed by Stokinger,[201] Veninga,[213] Menzel,[134] Zelac et al.[223] and others. As discussed in more detail later, Zelac et al.[223,224] have also demonstrated a nearly additive effect of ozone

and x irradiation in producing lymphocytic chromosomal breaks in Chinese hamsters. Furthermore, other antioxidant and radical-trapping agents—including various quinones, ascorbic acid, and α-tocopherol—have been reported to protect from ozone toxicity.[87,89,129,135,164,184]

The exact radicals produced by ozone *in vivo* are not fully understood. Alder and Hill[1] proposed that the aqueous decomposition of ozone produced both hydrogen and hydroxyl free radicals and that the reaction was catalyzed by base. Fetner[64] suggested that hydroxyl and peroxyl radicals were responsible for the observed radiomimetic effects. It should be noted that the peroxyl radical dissociates to form the superoxide anion radical at physiologic pH. Recently, Mustafa *et al.*[157] noted an increase in lung superoxide dismutase after ozone exposure, which indirectly suggested the presence of the superoxide anion radical.[201] Stokinger, noting the lability of sulfhydryl groups in the presence of ozone and radiation, hypothesized that free radicals might be derived from the interaction of ozone with sulfhydryls. A similar reaction pathway has been proposed by DeLucia *et al.*[45] That ozone-induced free radicals are derived from the oxidative decomposition of unsaturated fatty acids has been suggested by B. Goldstein and Balchum,[84] Menzel,[134] and Roehm *et al.*[185] B. Goldstein *et al.*[86] used electron paramagnetic resonance to demonstrate free-radical signals in ozonized linoleic acid. It is also conceivable that singlet oxygen is derived directly from ozone or via the decomposition of an ozonide. Furthermore, hydrogen peroxide, which may be formed in aqueous solution by ozone or these intermediates, has been indirectly identified in the red cells of animals that inhaled ozone.[82]

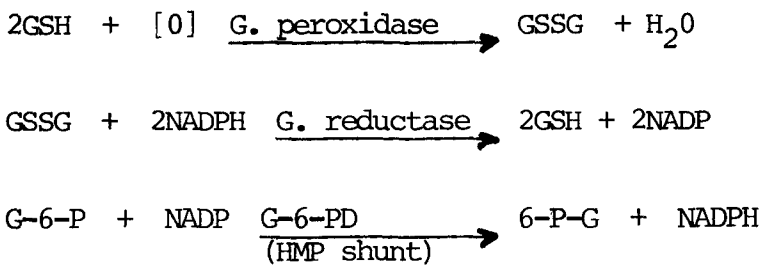
All these rapidly reacting intermediates are potentially harmful to the cell and might play a role in ozone toxicity. Furthermore, the potential for ozone-induced free-radical chain reactions exists. It appears likely that more than one radical is formed, either directly from ozone or as a result of the interaction of ozone with normal cellular constituents.

The implications of various oxidative free-radical reactions for biologic processes are being studied. Many of these reactions appear to play a role in normal cellular processes and may be of pathologic significance only if not contained by normal defense mechanisms. It is of interest that Shoaf et al.[194] have suggested on the basis of energetics that ozone might be derived from superoxide anion radical. Inasmuch as superoxide is formed during some intracellular processes, ozone might be a normal transient intermediate in vivo. Ozonides have also been produced in the absence of ozone by the photooxidation of diazo compounds in the presence of aldehydes.[155] Further information concerning the interrelationships of ozone, free radicals, peroxides, ozonides, and singlet oxygen in biologic systems would be useful.

Sulfhydryl Compounds and Pyridine Nucleotides: In view of the oxidant nature of ozone, a number of investigators have evaluated its effects on intracellular compounds that are normally active in cellular redox reactions. Attention has focused particularly on reduced pyridine nucleotides--reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide ademine dinucleotide phosphate (NADPH)--and on sulfhydryl compounds, specifically reduced glutathione (GSH).

Mudd,[143] Mudd et al.,[145] Menzel,[133] and Nasr et al.[161] have reported that ozonization of aqueous solutions of NADH or NADPH results in their oxidation. However, there is a difference in their findings as to whether the resulting product is a biologically active oxidized pyridine nucleotide (NAD or NADP), as suggested by Menzel,[133] or is molecularly disrupted to the extent that it is unable to participate in enzymatic processes. Inasmuch as more drastic effects are likely to be observed in vitro, it is more likely that oxidation of intracellular reduced pyridine nucleotides proceeds mainly to NAD or NADP after ozone inhalation, but further resolution of this question would be of value.

Nasr et al.[161] failed to observe a change in the ratio of NADPH to NADP in the tracheal epithelium of rats exposed to ozone at 33 ppm for an hour. This apparently negative in vivo finding is not surprising, inasmuch as NADP will be rapidly reduced back to NADPH if ozone does not disrupt the structural integrity of pyridine nucleotides. In addition, de novo synthesis of pyridine nucleotides may also occur. The intracellular ratio of reduced to oxidized pyridine nucleotides is under fine cellular control, in that the oxidation of NADPH or NADH results in the stimulation of enzymatic activity, which restores the initial ratio. In the case of NADPH, its oxidation increases the activity of the hexose monophosphate shunt; this also occurs after the oxidation of glutathione. The relevant enzymatic steps are depicted in Figure 8-1. The direct interrelationship of glutathione and NADPH occurs in the reduction of oxidized glutathione to reduced glutathione by glutathione reductase. NADPH is required in this step and is itself oxidized to NADP. Accordingly, the oxidation of



GSH=reduced glutathione

GSSG=oxidized glutathione

G. peroxidase=glutathione perioxidase

G. reductase=glutathione reductase

G-6-PD=glucose-6-phosphate dehydrogenase

6-PG=6-phosphogluconate

G-6-P=glucose-6-phosphate

[0]=oxidizing moiety, e.g., hydrogen peroxide, free radical, lipid peroxide

HMP shunt=hexose monophosphate shunt

NADP=nicotinamide adenine dinucleotide phosphate

NADPH=reduced nicotinamide adenine dinucleotide phosphate

Figure 8-1. Some enzyme processes active in defending against oxidant stress.

either NADPH or GSH conceivably accounts for the apparent increase in hexose monophosphate shunt enzymes after repeated ozone exposure.

de Koning and Jegier[44] also measured pyridine nucleotides in a study in which exposure of Euglena gracilis to ozone at 0.8 ppm for an hour in the presence of light produced a 9-12% decrease in NADH formation. Histochemical studies have demonstrated an alteration in NADPH- and NADH-diaphorase activity in the lungs of rats exposed to 0.8-ppm ozone for 7 days.[30]

A possible interaction of ozone with sulfhydryl groups in vivo was originally suggested by Fairchild et al.,[58,62] who noted that protection from lethal concentrations of ozone was conferred by the injection or inhalation of sulfhydryl or disulfide compounds. Other investigators have also noted protection from ozone toxicity by sulfhydryl compounds.[20,73,110,133] Mountain[142] observed a decrease in lung glutathione and in the activity of the sulfhydryl-containing enzyme succinic dehydrogenase after ozone exposure. The possible toxicologic implications of the reaction of ozone with sulfhydryl compounds were discussed in detail by Stokinger.[201]

Exposure of aqueous solutions of GSH to ozone results in the formation not only of GSSG, but also of higher oxidation states, including the sulfoxide.[133] This is important, in that, although the enzymes shown in Figure 8-1 are capable of recycling GSSG back to GSH, the production of the sulfoxide or the sulfone may lead to irreversible loss of glutathione. By analogy, a similar oxidation of protein sulfhydryl groups to the sulfoxide would also presumably prevent reduction by a disulfide-exchange mechanism with glutathione and therefore lead to irreversible

inactivation. However, the ozone-induced production of sulfoxides or sulfones has not been reported *in vivo*.

DeLucia *et al.*[45] reported that exposure of rats to 2-ppm ozone for 4-8 h resulted in a statistically significant decrease in both protein and nonprotein lung sulfhydryl groups. But, rats exposed to 0.8-ppm ozone for 10 days showed no change in lung sulfhydryl concentrations. As pointed out by the authors, this finding suggests lung adaptation and is consistent with their observation that chronic ozone exposure resulted in an increase in glucose-6-phosphate dehydrogenase (G-6-PD) concentration, despite a decrease in its activity after acute exposure. Glutathione reductase, and NADH and succinate cytochrome c reductase concentrations were also decreased after acute ozone exposure; this was ascribed to an effect on protein sulfhydryl groups. However, there is no firm evidence that free sulfhydryl groups are involved in the active sites of any of these enzymes in the lung. The cytochrome c reductases and glutathione reductases both contain flavins, which are relatively readily oxidized intermediates. Further studies on the sensitivity of flavin compounds to ozone appear warranted. In addition, there is evidence of an effect of ozone on cytochrome heme compounds, which might explain the decrease in cytochrome c reductase observed by DeLucia *et al.*[45]

More recently, DeLucia *et al.*[46] reported that the bulk of the glutathione oxidized in rat lung after exposure to 4-ppm ozone for 6 h was in the form of mixed disulfides with lung protein sulfhydryl groups. No increase in the concentration of oxidized glutathione was noted. Peak oxidation of nonprotein sulfhydryl groups did not occur until about 24 h

after exposure, and recovery was evident at 48 h. Effects of exposure to lower concentrations (0.8 ppm for 24 h and 1.5 ppm for 8 h) were not observed.

Further detailed studies of the effect of ozone on enzymes active in the defense against intracellular oxidation were performed by Chow and co-workers.[31-33] Chronic ozone exposure resulted in an increase in the activity of G-6-PD and 6-phosphogluconic dehydrogenase, two constituents of the hexose monophosphate shunt, and an increase in glutathione peroxidase and glutathione reductase concentrations (see Figure 8-1). Of particular note is a study in which groups of rats were exposed to ozone at 0.2, 0.5, or 0.8 ppm continuously for 8 days. The increases in G-6-PD, glutathione peroxidase, and glutathione reductase were linearly related to dose. Statistically significant differences were present after exposure to ozone at 0.2 ppm. In an additional study, exposure of rats to ozone at 0.75 ppm resulted initially in a decrease in the activity of these enzymes and then an increase, as exposure continued.[32] As discussed below, the authors related their findings to the action of glutathione peroxidase in detoxifying lipid peroxides, rather than to an ozone-induced intracellular aqueous radical or hydrogen peroxide. However, if the latter processes do occur, a similar increase in these enzymes after chronic exposure might be expected.

Lipids. Unsaturated fatty acids (UFA) are readily oxidized cellular macromolecules whose oxidative breakdown has been observed in a number of situations analogous to ozone toxicity, including radiation, exposure to hyperbaric oxygen,[132] and inhalation of nitrogen dioxide.[210] The classic

mechanism by which free radicals and oxidative states interact with the carbon-carbon double bonds of UFA is the formation of peroxides. Their decomposition may result in further free-radical production capable of initiating peroxidation of additional UFA. The resulting breakdown of the UFA molecule results in various addition and decomposition products, including peroxides and carbonyl compounds, which may themselves be toxic to the cell. Ozonization of UFA appears in many ways similar to lipid peroxidation in the resulting products and biologic implications. However, the initial attack on the double bond proceeds by a different mechanism. This has been studied in ozonized fatty-acid methyl ester emulsions and thin films by Roehm et al.,[184,185] whose findings support the general mechanism originally proposed by Criegee[40] (Figure 8-2). In this scheme, ozone directly attacks carbon-carbon double bonds; this results, after decomposition of the double bond, in the formation of a zwitterion and aldehyde. These recombine to produce the ozonide. As pointed out by Roehm et al.,[184] in the presence of water the zwitterion may form peroxides that can then catalyze the peroxidation of additional molecules of UFA. The ozone-induced oxidative decomposition of UFA in model membranes and emulsions has also been evaluated by Menzel et al.[135] and Teige et al.,[207] and the subject has been reviewed by Menzel.[134]

It is not clear from cellular and in vivo experiments whether the observed ozone-induced breakdown of UFA proceeds through the direct attack of ozone on UFA, through the effects of an ozone-induced free radical that result in lipid peroxidation, or through a combination of these processes. Inasmuch as phenolic antioxidants are not as effective

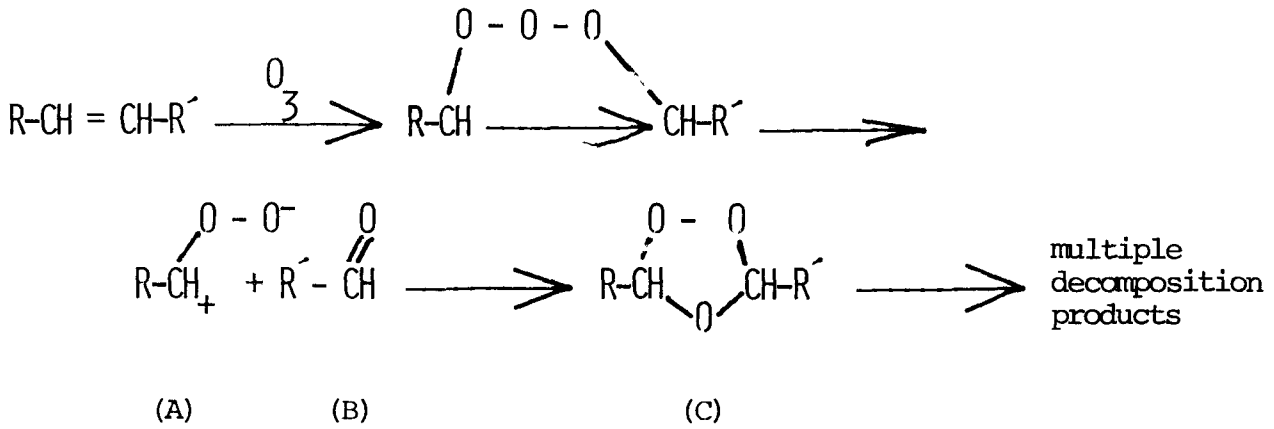


Figure 8-2. Mechanism of ozonolysis. A, Criegee zwitterion; B, aldehyde; C, ozonide.

in protecting against the direct attack of ozone on double bonds as they are in the autocatalytic lipid peroxidation process,[185] the finding that vitamin E protects against ozone toxicity might indirectly indicate that ozone-induced lipid peroxidation does occur.[136,137] However, Menzel et al., in a series of studies with preformed fatty-acid ozonides, have concluded that such compounds may be responsible for a significant proportion of ozone toxicity. Because "lipid peroxidation" is the term commonly used in the literature and the biologic implications of lipid ozonization and lipid peroxidation appear to be relatively similar, we will use the latter term in discussing the effects of ozone on UFA.

Evidence of a role of lipid peroxidation in the cellular toxicity of ozone has been obtained in in vitro studies in which human red cells were exposed to this oxidant gas.[9,84] The possibility that lipid peroxidation is responsible for altered permeability of bacterial cell walls after ozone exposure was proposed by Scott and Lesher[193] and has since been confirmed by others.[207] Of note is the study of Teige et al. in which red cells were lysed more readily by incubation with ozonized liposomes than they were by ozone itself.[137] Similarly, Menzel et al. noted that incubation of red cells in ozonized serum produced more toxicity than did direct ozonization of the red cells in the absence of serum. Menzel et al.[137] also demonstrated that incubation of human or mouse cells with fatty-acid ozonides produced Heinz bodies, methemoglobin formation, oxidation of thiol groups, and formation of mixed disulfides with hemoglobin. Oral administration of vitamin E protected against Heinz-body formation in vitro. Heinz bodies were also observed in the red cells of mice

exposed to ozone at 0.85 ppm for 48 h. Lung toxicity that mimicked that observed after ozone exposure has been seen by Cortesi and Privett[39] in rats that received intravenous injections of fatty-acid hydroperoxides or ozonides.

Lung lipid peroxidation during ozone inhalation was suggested by the finding of conjugated diene bonds in an extract of the lungs of mice exposed to ozone at 0.4-0.7 ppm for 4 h.[90] The presence of thiobarbituric acid reactants, predominantly malonaldehyde (another index of lipid peroxidation), has been observed in the lungs of rats exposed to ozone at 0.7-0.8 ppm continuously for 5-7 days.[33] The malonaldehyde concentration was linearly related to the concentration of glutathione peroxidase, and animals given α-tocopherol supplements did not have as great an increase in either malonaldehyde or glutathione peroxidase. The authors suggested that the increase in glutathione peroxidase concentration reflected the activity of this enzyme in detoxifying lipid peroxides, rather than a more direct ozone-induced intracellular radical or oxidizing species. This is a crucial point, particularly because in later studies there was no apparent threshold for the increase in glutathione peroxidases.[31] Lipid peroxidation is primarily a cell-membrane event, whereas GSH and its related enzymes are intracellular. Therefore, although an increase in these enzymes would presumably protect intracellular constituents against oxidation that is secondary to lipid peroxidation, direct protection of the cell membrane might not be expected. Accordingly, a lack of threshold for a lipid peroxidation-induced increase in glutathione peroxidase implies that any concentration of ozone produces lung lipid

peroxidation. However, such an interpretation must be viewed with caution, for at least two reasons: the assumption of linearity in the enzyme data is open to question, particularly because the findings are not inconsistent with a curvilinear function;[31] and lung lipid peroxidation at low concentrations of ozone (e.g., 0.2 ppm) has still not been documented, and it is possible that the observed enzyme increases, rather than reflecting lipid peroxidation, are in response to other intracellular ozone effects. If the latter is true, then the increase in glutathione peroxidase could be considered an adaptive response with no pathologic consequences to the lung, although this is open to debate. In this respect, it is of interest that Prinsloo[180] has reported an increase in lung hexose monophosphate shunt enzymes after inhalation of quartz dust.

Further indirect evidence of a role of lipid peroxidation in ozone toxicity has been obtained in studies in which animals deficient in vitamin E were found to be more susceptible to lethal concentrations of ozone and sublethal concentrations led to a more rapid utilization of this antioxidant vitamin.[87,135] Although vitamin E deficiency potentiates the effects of ozone, it is not completely clear whether supranormal concentrations of vitamin E protect against ozone toxicity. Mice given tocopherol supplements were not protected against lethal concentrations of ozone,[164] and the specific activity of lung hydrolases was found to be unrelated to dietary vitamin E concentration.[48] However, other investigators have reported that additional supplementation with vitamin E above usual dietary concentrations lessens the extent of toxicity in animals that inhale ozone.[31,33,159]

Mustafa et al.[158] were unable to detect lung lipid peroxides after short-term or subacute ozone exposures (0.8-2.0 ppm), but noted their presence after in vitro exposures of mitochondrial preparations. Dowell et al.[51] saw no evidence of lipid peroxidation in an alveolar macrophage preparation obtained from rabbits acutely exposed to ozone at up to 10 ppm.

The implications of an ozone-induced breakdown of UFA extend beyond the destruction of these integral components of cellular membranes. Lipid peroxides can produce hydrogen peroxide and oxidize sulfhydryl groups and other amino acid constituents, and this results in enzyme inactivation. In addition, lipid peroxidation has been implicated in one of the many theories that attempt to explain the aging process--that based in part on the apparent similarity of "normal" age pigments to the products of the reaction of lipid peroxides with proteins. In particular, proteins cross-linked by the dicarbonyl lipid peroxide breakdown product malonaldehyde have fluorescent spectra similar to that of lipofuchsin age pigment.[47] Bruch and Schlipköter[22] noted lipofuchsin granules in the lungs of mice exposed to ozone at 0.86 ppm for up to 10 months. Inasmuch as acceleration of aging has been reported after chronic ozone exposure,[191] it is conceivable that this is mediated through lipid peroxidation. In addition to free radicals and peroxides, the end products of the oxidative decomposition of UFA include carbonyl compounds that may also have toxic effects. Buell et al.[26] reported the presence of carbonyl compounds in the lungs of rabbits exposed to ozone at 1 ppm for 1 h and suggested that these compounds can cross-link collagen or elastin and so lead to altered lung function. Increased cross-linking of collagen has also been suggested as a mechanism

of the deterioration of lung function with age. Recently, Sugihara and Martin[205] reported simulation of lung aging after exposure to formaldehyde, a cross-linking agent.

Proteins. As described above, sulfhydryl groups are relatively susceptible to oxidation by ozone, and a decrease in lung-protein sulfhydryl groups has been reported as a consequence of ozone exposure. Free sulfhydryl groups are important constituents of many enzymes, and oxidation results in a loss in activity. In addition, cell-membrane sulfhydryl groups play an important role in the transport of water and ions, and it is conceivable that their oxidation in the lung is a factor in ozone-induced pulmonary edema.

The oxidation of cysteine, as well as other amino acids, was studied by Mudd et al.[144] Individual amino acids in aqueous solution were exposed to ozone; the reported order of susceptibility was cysteine, methionine, tryptophan, tyrosine, histidine, cystine, and phenylalanine. Other amino acids were not affected. This order is similar to that for the relative susceptibility of amino acids to radiation and to lipid peroxides. Evaluation of the ozonization products revealed that cysteine was converted to cysteic acid, as well as cystine; methionine to methionine sulfoxide; tryptophan to a variety of products, including kynurenine and N-formylkynurenine; tyrosine also to a variety of products, including dihydroxyphenylalanine; histidine to ammonia, proline, and other compounds; and cystine in part to cysteic acid. In some cases, the rate and end products depended on the pH of the solution.

Somewhat different oxidation products were observed by Previero et al.,[178,179] who performed the ozonization in anhydrous formic acid, rather than aqueous solution. This raises the possibility that *in vivo* effects on protein may depend on the relative hydrophobicity of the location of the susceptible amino acid, particularly in cell-membrane lipoproteins.

A number of investigators have studied the effect of ozone on the ultraviolet absorption spectra of proteins and amino acids. A decrease in the absorption of 280-nm light in a number of proteins was originally reported by Giese et al.[79] to be a consequence of ozone exposure; they suggested that this was due to an interaction of ozone with the ring structures of tyrosine and tryptophan. Exposure of a solution of tryptophan to ozone resulted in a decrease in 280-nm absorption, whereas the extinction coefficient of tyrosine increased. Similar results with tyrosine were reported by Scheel et al.,[191] who also noted alterations in the ultraviolet spectra of egg albumen, perhaps representing denaturation by ozone.

More recently, B. Goldstein and McDonagh[91] demonstrated that the native protein fluorescence (280-nm excitation, 330-nm emission) of red-cell membranes exposed *in vitro* to ozone at 1 ppm was a somewhat more sensitive indicator of ozone effect than other characteristics measured in the same system, including oxidation of cell-membrane sulfhydryl groups, loss of acetylcholinesterase activity, and formation of lipid peroxide breakdown products.

Among the specific enzymes whose activity has been reported to be decreased after *in vitro* ozone exposure are papain,[133] glyceraldehyde-3-phosphate dehydrogenase,[107] lysozyme,[144] ribonuclease,[83,167] and acetylcholinesterase. The latter enzyme appears to be particularly susceptible to free-radical and oxidative states. A loss in acetylcholinesterase activity has been reported in the red cells of humans[23] and mice[92] that inhaled ozone. However, there are only minimal amounts of this enzyme in lung tissue, and, although it has been suggested that acetylcholinesterase is important in bronchial tract ciliary activity,[26] there is no direct evidence to support this conjecture.

As described elsewhere in this chapter, alterations in the activity of a number of lung enzymes have been described after acute and chronic ozone exposure. With the possible exceptions of the sulfhydryl-containing enzyme succinic dehydrogenase and the cytochrome P-450 enzyme benzopyrene hydroxylase, it is difficult to determine whether these findings are due to a direct oxidative effect of ozone or are secondary to changes in protein synthesis, concentrations of intermediates, or destruction of cells or organelles.

Nucleic acids. Less is known about the interaction of ozone with other macromolecules. Alterations of nucleic acids in solution have been reported by Christensen and Giese,[34] and in *Escherichia coli* by Prat *et al.*[177] in studies with relatively high concentrations of ozone. Both purines and pyrimidines are attacked by ozone. However, the detailed chemistry of the reaction, including the intermediates and end products, has not been fully elucidated. This may be important, in view of the

chromosomal effects of ozone, although such effects are most likely due to the interaction of an ozone-induced intermediate with DNA, rather than to ozone itself.

Studies of DNA synthesis in lung after ozone exposure have yielded varied results. Scheel et al.[191] exposed rats to ozone at 9.2 ppm for 45 min and reported an initial decrease in lung DNA content, which returned to normal in 6 h. In contrast, lung RNA content was found to increase sharply (as fraction of body weight) an hour after exposure. Cronin and Giri[41] found no change in DNA content per lung in rats exposed to ozone at 4 ppm for 4 h. Evans et al.[56] used a more sensitive autoradiographic procedure and reported a decrease in the fraction of mouse lung cells that synthesized DNA immediately after exposure to ozone at 0.5-3.5 ppm for 6 h. DNA synthesis had returned to normal or above normal within 72 h after exposure. More recently, Werthamer et al.[219] studied the uptake of tritiated thymidine, uridine, and leucine in lung homogenates obtained from mice exposed to ozone at 2.5 ppm for 2 h/day for up to 120 consecutive days. Both DNA and RNA synthesis initially declined, but protein synthesis was greatly increased. By 30 days of exposure, DNA synthesis was above normal, but RNA synthesis remained low.

Carbohydrates. Even less is known about the effects of ozone on carbohydrates. Buell et al.[26] observed a decrease in the depolymerization of hyaluronic acid after treatment of the lungs of ozone-exposed rabbits (1 ppm for 1 h) with hyaluronidase. B. Goldstein et al.[88] reported a loss in membrane neuraminic acid of red cells exposed in vitro to high concentrations of ozone. It would be important to study the effects of

ozone on respiratory tract mucus, which is rich in carbohydrates, including neuraminic acid. This could include determination of the extent to which ozone is able to penetrate mucus that is unreacted, whether the reaction of ozone with mucus results in the formation of cytotoxic intermediates, and evaluation of the interaction in mucus of ozone with other air pollutants, particularly sulfur dioxide. Of possible pertinence is a study by Falk et al.,[63] who observed that ozone produced a loss in the viral hemagglutinating ability of snail mucus.

Subcellular Components

Current approaches to understanding the biochemistry of organ systems depend heavily on fractionation procedures designed to separate various cellular and subcellular components. The lung is a very complex organ, which contains many cell types that presumably function in different ways and may well have different key biochemical processes. Unfortunately, the lung is difficult to homogenize and fractionate, and this, along with the multiplicity of cell types, has greatly retarded understanding of normal lung biochemistry.

Many studies have evaluated the biochemical effects of ozone and other pollutants with whole-lung homogenates. This is a somewhat unsatisfactory approach, inasmuch as a toxic effect at a specific target site may be obscured by reciprocal adaptive changes in other pulmonary cells or organelles, as well as by an influx of leukocytes and the presence of edema. Of interest in this regard is the observation of an ozone-induced increase in the number of lung Clara cells[22,170] and a shift from type 1 to type 2 pneumocytes, which has been hypothesized

to be related to antioxidant defense.[42] The biochemical concomitants of these morphologic alterations are unknown and are unlikely to be ascertained by using whole-lung homogenates. For a full understanding of the effects of ozone in the lung, and in particular for a determination of the lowest ozone concentrations that affect specific important indexes of toxicity, more information on normal lung biochemistry is necessary.

In general, the lung has been considered to be the major site of ozone toxicity, if for no other reason than because it is the first organ to come into contact with inhaled ozone. It is therefore of interest that, in a study by Cortesi and Privett,[39] intravenous injection of a fatty-acid ozonide in rats resulted in lung edema and hemorrhage and that on gross examination the lung was the only organ noted to be affected. Similar results were observed after injection of a fatty-acid hydroperoxide.[206] Other systemic agents that are relatively specific to the lung and whose toxicity is perhaps mediated by free-radical reactions include paraquat[118] and 3-methylindole.[28] These findings raise the possibility that there is some unknown biochemical aspect of the normal lung that, in comparison with other organs, makes it relatively susceptible to oxidative free-radical-including agents like ozone.

Membranes. The possibility that pulmonary membranes are a primary site of ozone toxicity is suggested by a number of lines of evidence, most of which are indirect. These include observations that the membrane is the major site of ozone toxicity in plants[171] and bacteria;[193] morphologic evidence of pulmonary membrane damage after ozone exposure in a number of studies; and *in vitro* experiments with human red cells and artificial lipid membranes.

As discussed above, UFA which are present primarily in cellular membranes, appear to be particularly susceptible to oxidative degradation by ozone. Various studies of membrane lipid peroxidation have implicated this process in damage to organelles, including mitochondria, microsomes, and lysosomes, as well as to the cell membrane itself. By analogy, it is conceivable that many of the findings in cells and subcellular components described in other sections of this chapter are secondary to ozone-induced lipid peroxidation. However, this remains conjectural. Furthermore, the effects of ozone on the cell membrane do not appear to be limited to UFA. Protein is also affected, particularly cell-membrane sulfhydryl groups and aromatic amino acids. Whether these protein effects are secondary to cell-membrane lipid peroxidation or arise as a direct result of ozone is not clear.

No studies of the effect of ozone on a lung plasma membrane fraction have been reported. However, a few studies have evaluated the activity in whole-lung homogenates of enzymes that are believed to be associated with membranes. Scheel et al.[191] reported a decrease in both 5'-nucleotidase and alkaline phosphatase concentrations (expressed as amount per milligram of tissue) in rats exposed to ozone at 16.9 ppm for 1 h or 6 ppm for 4 h. Werthamer et al.[219] exposed mice to ozone at 2.5 ppm for 2 h/day on consecutive days. No effect on the concentration of alkaline phosphatase in lung homogenates was observed after the first day of exposure, and enzyme activity rose after 3 days of exposure. This was followed by a precipitous decrease in alkaline phosphatase activity (expressed as amount per milligram of lung protein) to about 50% of

control values, which persisted for 30 consecutive days of exposure. Cronin and Giri[41] reported a decrease in lung adenosine triphosphatase (ATPase) concentration (expressed as fraction of total lung weight) in rats exposed to ozone at 4 ppm for 4 h. However, this appeared to be due to the edema, inasmuch as recalculating the data in relation to total DNA revealed no difference between control and ozone-exposed lung ATPase concentrations.

Microsomes. An effect of ozone on lung microsomes has been suggested by morphologic studies that indicated alterations in the endoplasmic reticulum.[11,75,108] Biochemical evidence of an effect on microsomal enzymes was originally obtained in the studies of Palmer et al.,[166] who demonstrated that ozone exposure (0.75-10 ppm for 3 h) resulted in a decrease in activity of Syrian hamster lung benzopyrene hydroxylase, a mixed-function oxidase that depends on cytochrome P-450. No change in hepatic activities of this enzyme were observed, and the results were similar in animals in which high activities of benzopyrene hydroxylase had been induced. The maximal effect was not observed until a few days after the single ozone exposure. Palmer et al.[165] also reported a decrease in rabbit tracheobronchial mucosal benzopyrene hydroxylase activity after exposure to similar ozone concentrations.

Recently, B. Goldstein et al.[93] observed a decrease in microsomal cytochrome P-450 concentrations in the lungs of rabbits exposed to ozone at 1 ppm for 90 min. Again, the nadir of the cytochrome P-450 was not reached until a few days after ozone exposure. B. Goldstein and Balchum[85] had previously obtained indirect evidence that microsomal cytochrome P-450

played a role in the expression of ozone toxicity. Rats given inducing regimens of phenobarbital, which are reported to increase cytochrome P-450, were more susceptible to lethal concentrations of ozone; but injection of allylisopropylacetamide, which destroys cytochrome P-450, resulted in protection. The finding that mixed-function oxidase inhibitors protect plants against ozone injury[119] may be analogous. Other known free-radical-trapping agents shown to protect against ozone toxicity have often been given in multiple daily doses before acute ozone exposure and may also be active through alteration of microsomal intermediates. However, lung cytochrome P-450 concentrations have not been measured in conjunction with any of these agents.

Liver cytochrome P-450 has been shown to be destroyed by lipid peroxides,[190] and *in vitro* exposure of lung microsomes to ozone is accompanied by a loss in cytochrome P-450 and the formation of malonaldehyde.[93] Evidence of a possible effect of inhaled ozone on hepatic microsomes has been obtained by Gardner *et al.*,[76] who demonstrated that mice exposed to ozone at 1 ppm for 3 h on 2 or 3 successive days developed a prolongation of pentobarbital sleeping time. No effect was observed immediately after exposure on the first day, nor was there any significant difference from the control after 4-7 days of exposure. The latter finding appeared to be due to tolerance, inasmuch as increasing the dose to 5 ppm after 7 days at 1 ppm again resulted in a prolongation of pentobarbital sleeping time. The authors suggested that their findings reflect a decrease in hepatic microsomal metabolism of pentobarbital, perhaps due to an oxidizing circulating intermediate.

Mitochondria. Numerous ultrastructural studies have noted lung mitochondrial swelling and degenerative changes after ozone exposure.[11,15,16,170,174-176,192,197,198] Mitochondrial swelling occurs *in vitro* in association with lipid peroxidation of the mitochondrial membrane. However, the biochemical concomitants of ozone-induced damage to lung mitochondria have not been extensively studied.

Freebairn[73] noted a decrease in oxygen uptake of plant and bovine liver mitochondria that was reversible by glutathione and ascorbic acid. The activity of some mitochondrial enzymes, including succinic dehydrogenase and cytochrome oxidase, has been found to be susceptible to ozone.

Mustafa and colleagues have performed a series of studies on the effect of ozone on rat lung mitochondrial oxygen consumption. Acute-exposure studies (2 ppm for 8 h and 3 ppm for 4 h) revealed that an initial decrease in succinate dependent oxygen consumption was followed by a rebound in which increase in the utilization of succinate, as well as other substrates, was noted.[156,158] This increase persisted for at least 3 weeks. No difference in the yield of mitochondria between control and exposed lungs was noted immediately after ozone exposure, but there was a definite increase in the number of mitochondria in the exposed lungs within 48 h of recovery. The authors concluded that increased lung mitochondrial oxygen consumption during recovery from ozone was due to both enzyme activation and mitochondrial proliferation, but primarily the latter. Continuous exposure to ozone at 0.8 ppm for 10 or 20 days resulted in a significant increase in lung-homogenate oxygen consumption. In a further experiment, rats were exposed continuously for 7 days to

ozone at 0.2, 0.5, or 0.8 ppm.[158] The observed increase in oxygen consumption was statistically significant at all concentrations of ozone and varied directly with dose.

More recently, Mustafa[159] studied succinate-dependent mitochondrial oxygen consumption in rats fed either a diet containing vitamin E at approximately the normal American dietary intake or one with 6 times as much vitamin E. A statistically significant increase in oxygen consumption was observed in rats that received the diet lower in vitamin E when exposed to ozone at either 0.1 or 0.2 ppm for 7 days. In rats on the diet higher in vitamin E, ozone at 0.2 ppm for 7 days also produced a significant increase, but ozone at 0.1 ppm was without effect. Unfortunately, serum vitamin E concentrations were not given.

Lysosomes. The effect of ozone on lysosomal enzymes has been studied by a number of investigators. Ozone has been clearly shown to inactivate lysozyme *in vitro*, but the effects of inhaled ozone on the activity of lysozyme appear to depend on the pulmonary fraction under study. Holzman *et al.*[107] reported that exposure of rabbits or mice to ozone resulted in a decrease in the lysozyme activity of bronchopulmonary lavage samples. The effect was linearly related to the product of ozone concentration and duration of exposure, although relatively high concentrations of ozone (2.0-5.5 ppm) were used in this acute-exposure experiment (1-4 h). The authors also reported that alveolar cells present in the bronchial lavage of rabbits exposed to ozone at 10 ppm for 3 h had a decrease in the rate of lysosomal release when incubated *in vitro*. The inference that this is due to *in vivo* oxidation of intracellular lysozyme

is questionable, in view of the different cell populations present in the ozone-exposed and control bronchial lavage specimens. Of interest, however, is a reported decrease in the release of lysozyme from a peritoneal polymorphonuclear leukocyte sample obtained from the ozone-exposed rabbits.

In further studies by the same group,[4,111] depression in the activity of lysosomal enzymes--acid phosphatase, β-glucuronidase, and lysozyme--was observed in alveolar macrophages obtained from rabbits exposed to ozone at as low as 0.25 ppm for 3 h. Enzyme concentrations returned toward normal 24 h after exposure. An ozone-induced redistribution of lysosomal enzyme into the cytosol was suggested by a fractionation study that demonstrated a smaller fraction of enzyme activity in the alveolar macrophage lysosomal pellet, compared with the supernatant, with increasing doses of ozone.[110] *In vitro* studies of rabbit alveolar macrophages in tissue culture revealed an ozone-induced decrease in lysosomal acid hydrolase activity, which was prevented when sulfhydryl compounds or serum were added to the tissue-culture medium. A decrease in lysozyme has also been reported in the tears of humans reacting to photochemical smog.[189]

In contradistinction to the decrease in lysosomal enzyme activity observed in alveolar macrophages and bronchial lavage fluid, Dillard *et al.*[48] reported that continuous ozone exposure (0.70-0.79 ppm for 5-7 days) resulted in an increase in the activity of some lysosomal hydrolases in rat whole-lung homogenates and lung fractions, including the soluble supernatant. The tocopherol concentrations of the diet had no effect on the findings.

Similarly, Chow et al.[31] observed an increase in the lysozyme activity of a soluble lung fraction and of plasma after continuous exposure of rats to ozone at 0.8 ppm for 8 days. However, no difference in lung or plasma lysozyme activity from control values was present in rats continuously exposed to 0.2 or 0.5 ppm or intermittently exposed (0.2-0.8 ppm, 8 h/day for 7 days). Histochemical evidence of an increase in lung acid phosphatase, a lysosomal enzyme, has also been reported.[29]

The apparently conflicting data on lysozyme may perhaps be explainable by considering three distinct effects of ozone: an irritative response that produces an influx into the lung of leukocytes, including the alveolar macrophage, which contain lysozyme at relatively high concentrations, thereby leading to an increase in whole-lung lysozyme concentration; a disruption of lung lysosomal membranes, resulting in the release of acid hydrolases; and oxidation of lysosomal protein, producing enzyme inactivation. Evidence to support each of these processes is available, and it appears that the net observed effects will depend on the ozone dose and the lung fraction that is evaluated.

As discussed in detail by Dillard et al.[48] and by Mittman et al.,[141] the possible relationship of lysosomal proteases to chronic lung disease has been inferred from the finding of an increased incidence of emphysema in subjects deficient in serum α_1-antitrypsin factor, an α_1-globulin that can inhibit lysosomal proteases. (No effect of ozone on serum α_1-antitrypsin inhibitor was noted in rabbits chronically exposed to ozone.[168]) Thus, an ozone-induced increase in concentrations of such enzymes in the lung might produce excess proteolysis and result in eventual chronic lung disease. However, the available evidence is inadequate to support the belief that such a process

occurs in humans intermittently exposed to ozone that results from photochemical smog formation. Further studies of this potential hazard would be of value.

Connective Tissue. Despite physiologic evidence that chronic ozone exposure may affect lung elasticity,[10] there is very little information concerning the biochemical effects of ozone on lung collagen. Buell et al.[26] obtained a collagen-containing fraction from the lungs of rabbits exposed to ozone at 1 ppm for 1 h. A number of dinitrophenylhydrazine-reacting carbonyl compounds were obtained after digestion with collagenase or elastase. The authors suggested that such carbonyls might result in intramolecular or intermolecular cross-linking of collagen with an attendant decline in lung function. On the basis of present information, these carbonyl compounds are most likely derived from lipid.

In a recent abstract, Hussain et al.[112] presented evidence that exposure of rats to ozone at 0.8 ppm for up to a week resulted in an increased rate of collagen synthesis. Such a finding might be relevant to ozone-induced fibrosis. Obviously, further study of the biochemical effects of ozone on lung collagen and elastin are in order, particularly in conjunction with chronic-exposure experiments.

Alveolar Macrophages

Alveolar macrophages represent a major line of pulmonary defense against exogenous material, including infectious agents and particles. Although the origin of these phagocytic cells is still not completely clear, recent information suggests that they are derived from the bone marrow,[24] but are capable of further division and replenishment in the

lung.[81] As discussed below, a number of investigators have reported alterations in alveolar macrophage function and biochemistry after ozone exposure. Before a description of their observations, one potential difficulty in interpreting the effect of ozone inhalation on alveolar macrophages should be mentioned. Inhalation of ozone or other irritants results in an alteration of the number of alveolar macrophages obtained in a bronchial lavage. Presumably, this is due to a combination of cell destruction and an influx of younger alveolar macrophages in response to the irritant stress. It is well known that immature human granulocytes have functional capacities and concentrations of biochemical intermediates different from those of mature polymorphonuclear leukocytes. Unfortunately, there is no readily available morphologic indicator of alveolar macrophage maturity, as there is for granulocytes. Accordingly, observed differences between alveolar macrophages obtained after ozone exposure and those present in normal lungs may simply reflect relative immaturity secondary to pulmonary irritation, rather than a direct effect of ozone on biochemistry or function.

Interest in the effects of ozone on alveolar macrophages has been spurred by the observation that relatively low concentrations of ozone potentiate respiratory infections in animals and perhaps man. Coffin et al.[37] observed a decrease in the number of bacteria phagocytized by alveolar macrophages obtained from rabbits exposed to various concentrations of ozone as low as 0.3 ppm. Some suggestion of a lack of threshold is present, but it is not clear whether the difference from the controls at lower ozone concentrations is statistically significant.

These findings in rabbits are similar to those of E. Goldstein et al.,[94-97] who have related increased bacterial infectivity in mice to an ozone-induced impairment in the bactericidal capabilities of alveolar macrophages. They used radioactively labeled bacteria and observed a decrease in mouse lung bactericidal activity after a 4-h exposure to ozone at about 0.3-0.4 ppm. With this technique the effects of ozone and nitrogen dioxide are roughly additive,[97] and silicotic mice are no more susceptible to ozone than are control mice.[94]

Dowell et al.[51] reported an increase in the osmotic fragility of alveolar macrophage preparations obtained from rabbits acutely exposed to ozone at 10 ppm for 3 h or intermittently exposed to ozone at 2 ppm for 8 h/d for 7 days. Similar intermittent exposure to ozone at 0.5 ppm was without effect. A test for malonaldehyde formation was negative, but this lipid peroxide breakdown product may have been lost during the preparatory procedure.

Additional studies related to the alveolar macrophage include that of Weissbecker et al.,[218] who demonstrated that in vitro exposure to ozone at as low as 0.06 ppm resulted in alveolar macrophage cell death. However, Richmond[183] observed only a slight decrease in bacterial phagocytosis during in vitro incubation of alveolar macrophages with an initial ozone concentration of 0.8 ppm. Inasmuch as ozone was not administered continuously, the dose to the cells may have been minimal.

Gardner et al.[78] noted the ozone-induced loss of an alveolar macrophage protective component in lung lavage fluid after acute ozone exposure (10 ppm). This factor appears to stabilize alveolar macrophages

in lung fluid, and its loss would result in a decrease in cell viability. As pointed out by the authors, this finding might indicate that the reported effects of ozone on alveolar macrophages are, at least in part, due indirectly to an action on an alveolar fluid component, rather than on the macrophage itself. The substantial decrease in recoverable alveolar macrophages after ozone inhalation does suggest that ozone has a direct effect on alveolar macrophages, as does the study of Huber et al.,[108] who noted nonspecific ultrastructural alterations in rabbit alveolar macrophages after exposure to ozone at 5 ppm for 3 h. However, other investigators have failed to observe morphologic abnormalities in alveolar macrophages after inhalation of ozone.[16,176]

Mechanisms by which ozone might interfere with bactericidal function include an alteration in cell-membrane function that produces a loss in phagocytic ability and is perhaps mediated by lipid peroxides[116] and a decrease in the ability of alveolar macrophages to kill phagocytized bacteria. Leukocytes in general appear to have a multiplicity of mechanisms for destroying ingested bacteria.

Ozone might interfere with the intracellular bactericidal capabilities of alveolar macrophages by inactivating lysosomal hydrolases, or perhaps through the destruction of heme-containing enzymes that are apparently involved in producing superoxide anion radical. Further evaluation of the process by which relatively low concentrations of ozone potentiate bacterial infection would be of value.

Extrapulmonary Effects

An appreciable body of evidence has accumulated to indicate that ozone has extrapulmonary effects. Although some of the reported effects may be secondary to the reaction of ozone with intrapulmonary neural receptors or to release of humoral substances from the lung, other findings appear to be more directly related to an oxidizing effect of ozone. The biochemical basis for the latter is unclear, particularly because the reactivity of ozone and its short-lived intermediates would make it unlikely for them to penetrate the pulmonary parenchyma. Earlier studies on the subject of extrapulmonary effects have been reviewed by Stokinger.[201]

Industrial and epidemiologic evidence associating ozone exposure with headache and drowsiness are relatively nonspecific and do not clearly indicate extrapulmonary effects. Lagerwerff[124] noted changes in a number of indexes of vision at ozone concentrations of 0.2-0.5 ppm during short-term exposures. In addition, Brinkman and Lamberts[20] reported a 50% decrease in the rate of desaturation of oxyhemoglobin in the skin capillaries of a tied-off finger during inhalation of ozone at 1 ppm (the experimental procedure was not given in detail). Neither of these studies, which have been in the literature for some time, has been replicated.

Recently, Buckley et al.[23] have described statistically significant changes in a number of constituents and properties of red cells and serum in human volunteers experimentally exposed to ozone at 0.5 ppm for 165 min. Their findings included an increase in red-cell osmotic fragility

and a decrease in red-cell acetylcholinesterase and glutathione, all of which were consistent with previous *in vitro* or animal studies. They also observed an increase in serum thiobarbituric acid reactants consistent with lipid peroxidation and an increase in serum vitamin E that they attributed to its mobilization. The thiobarbituric acid assay is predominantly a measure of malonaldehyde. Increased serum malonaldehyde concentrations have usually been reported in man only in association with significant red-cell lipid peroxidation, such as occurs in hemolytic vitamin E deficiency, presumably because malonaldehyde is rapidly metabolized and cleared from the serum. Also consistent with acute hemolysis is the observation by Buckley *et al.* in the same subjects of increases in serum lactic dehydrogenase and in red-cell G-6-PD. In contrast with those in the lung, G-6-PD increases in the anucleate red cell are thought to represent solely a decrease in mean red-cell age consistent with reticulocytosis, which (to explain the extent of the G-6-PD increase in this study) would be consistent with a relatively significant acute hemolysis. Data necessary to interpret this possibility were not fully presented.[99] A more recent report from this group evaluating dose-response data for human 2-h exposures to ozone at 0.25, 0.37, and 0.50 ppm showed a no-effect concentration of 0.25 ppm for mean change in red-cell acetylcholinesterase activity and osmotic fragility and a linear dose-related effect on these two characteristics at the higher ozone concentrations.

A number of animal studies have revealed extrapulmonary effects. Again, there is some question as to which of these may represent an effect

of ozone, or a direct ozone-induced intermediate, rather than a more indirect response to pulmonary toxicity, perhaps mediated by neurohumoral factors. Thus, for instance, the observations of altered hepatic nucleic acid concentrations,[191] shifts in the content of metals in the liver,[50] alterations in urinary pH,[102] increases in liver weight and alkaline phosphatase activities,[148] and variations in the circulating white-cell count[17] in ozone-exposed animals conceivably represent nonspecific responses to lung injury.

Of interest is the experimental approach whereby ozone is delivered solely to one lung. The observation of pulmonary effects in the unexposed lung[71] indicates that there are extrapulmonary effects of ozone at edematogenic concentrations. However, only the exposed lung appears to develop tolerance to later ozone exposure and to exhibit impairment of bacterial defense mechanisms.[2,3,72]

Evidence of a direct oxidizing effect on the blood of mice and rats inhaling ozone was obtained in a study in which an increase in an *in vivo* red-cell catalase-reacting compound, presumably hydrogen peroxide,[82] was noted. Although this was observed only at ozone concentrations of 5 ppm or greater, the indirect assay for red-cell hydrogen peroxide used in this study is relatively insensitive and would not detect smaller degrees of ozone-induced oxidant stress. A significant decrease in the activity of red-cell acetylcholinesterase, a membrane constituent known to be sensitive to lipid peroxides, has been reported in mice exposed for 4 h to ozone at 8 ppm.[92] However, only a slight decrease in red-cell acetylcholinesterase was noted in rabbits chronically exposed to ozone

at 0.4 ppm.[114] An earlier study by Brinkman et al.[21] noted that 30-60 min of inhalation of ozone at 0.2 ppm potentiated the sphering of animal and human red cells irradiated in vitro. The contention that this intriguing effect is related to an increase in aging by ozone is open to question. Ozone effects in blood also include chromosal aberrations in the circulating lymphocytes of hamsters,[223,224] an increase in plasma lysozyme,[31] higher serum concentrations of trypsin protein esterase,[169] and Heinz bodies in circulating red cells.[136,137] Earlier studies by Mittler et al.[140] failed to reveal any change in the hemoglobin or hematocrit of young rats chronically exposed to ozone at 2.4 ppm.

Biochemical changes in animal central nervous systems have been reported by Skillen et al.,[195] who noted a decrease in brain 5-hydroxytryptamine (serotonin) in rats exposed to ozone at 6 ppm for 4 h, and by Trams et al.,[211] who observed decreases in catecholamines and catechol-O-methyltransferase in dogs chronically exposed to ozone at 1, 2, or 3 ppm. Electroencephalographic (EEG) measurements in the same dogs were recently presented by Johnson et al.,[115] who noted alterations in EEG patterns at 9 months of ozone exposure, but not after 18 months of exposure. Previously, Xintaras et al.[221] had observed alterations in the visual evoked electric response in rats acutely exposed to 0.5-1.0 ppm.[115] As pointed out by Johnson et al., it is not clear whether these findings indicate a direct neurotoxic action of ozone or are secondary to damage in other organs.

Other reported systemic effects of ozone include histologic changes in the parathyroid glands of rats exposed to ozone at 0.75 ppm for 4-8 h,[8]

variations in rat thyroid function that depend on the concentration and duration of ozone exposure,[61] alterations in myocardial histology in mice exposed to 0.2 ppm 5 h/day for 3 weeks,[21] ECG changes in dogs chronically exposed to irradiated auto exhaust (containing oxidants at 0.4-0.8 ppm),[14] inhibition of gastric motility in rats exposed to ozone at 0.5 ppm for 2 h,[186] and a decrease in the release of lysozyme from peritoneal granulocytes in ozone-exposed rabbits.[107]

An interesting report by P'an and Jegier[169] noted an increase in serum trypsin protein esterase in association with pulmonary vascular lesions in rabbits chronically exposed to ozone at 0.4 ppm. This serum α-2-macroglobulin, which is synthesized in the liver, has been reported to be increased in human vascular disorders, but its physiologic significance is unknown.

In summary, the current evidence appears to support the contention that ozone or an oxidizing derivative is able to penetrate the alveolar basement membrane into the pulmonary circulation. Recent evidence, which requires replication, suggests that red-cell changes consistent with an oxidizing effect can be detected in the blood of humans exposed to ozone at ambient concentrations. There is also evidence that ozone affects other extrapulmonary organ systems, but it is not clear whether this is due to oxidizing intermediates--such as ozonides, peroxides, and the resulting carbonyl compounds carried in the circulation--or whether it is secondary to reflex arcs or to humoral processes initiated in the lung.

MUTAGENIC AND CARCINOGENIC ACTIONS

The supposition that ozone is mutagenic or carcinogenic in man is based primarily on information on the biochemical mechanism of ozone toxicity and to a lesser extent on *in vitro* and animal studies. The biochemical evidence is for the most part indirect and depends on an analogy between the free-radical nature of ozone toxicity and of radiation and other carcinogenic agents.

The production of chromosomal abnormalities by ozone was first observed in plants by Fetner,[64] who noted abnormal anaphases in the root tips of *Vicia fava* seeds exposed to ozone. Bacterial mutagenesis by ozone has been reported by a number of investigators.[43,214] Hamelin and Chung[100,101] noted an increased mutation rate in *Escherichia coli* exposed to ozone at as low as 0.05 ppm for 5 min and observed that the mutants were sensitive to ozone and x irradiation. They suggested that ozone might interact in DNA repair processes. Other studies indicating ozone-induced chromosomal effects include the observation of mitotic inhibition in grasshopper neuroblasts[66] and abnormalities in the eggs of oysters treated with ozonized seawater.[126]

Fetner[65] has also demonstrated chromatid breaks in a human tissue-culture cell line exposed to ozone at 8 ppm for 5 min. Other tissue-culture studies include that of Sachsenmaier *et al.*,[187] who noted tetraploidy and other chromosomal abnormalities in embryonic chick fibroblasts exposed to ozone and a decrease in transplantability of mouse ascites tumor cells. In addition, Pace et al.[163] demonstrated an interference by ozone with mitotic activity in two tissue-culture cell

lines. More recently, Booher et al.[19] reported that lung cells exposed in culture to ozone concentrations as low as 0.3 ppm demonstrated an inhibition in growth that was proportional to the ozone concentration.

Of particular interest are the studies of Zelac et al.[223,224] in which Chinese hamsters exposed to ozone at 0.2 ppm for 5 h had an increased number of chromosomal breaks in their circulating lymphocytes. Blood samples for study were obtained immediately after exposure and 6 and 15.5 days later. The highest break frequency was observed after the longest delay. The authors compared the effects of x irradiation and ozone singly and combined, in their system. The combined effects were less than additive; this suggested some protective mechanism, perhaps analogous to that observed by Hattori et al.[103] When the authors extrapolated their data to acceptable industrial-hygiene exposures to ozone and radiation, ozone was found to be much more likely than x irradiation to produce chromosomal breaks in such exposures.

More recently, Mertz et al.[138] has studied the circulating lymphocytes of humans experimentally exposed to ozone at 0.5 ppm for 6-10 h. A statistically significant increase in the number of minor chromosomal abnormalities (not breaks) was observed; it reached a peak about 2 weeks after exposure and later returned to normal. This delay in the development of chromosomal abnormalities observed after ozone exposure in both hamsters and humans differs from that observed in human radiation studies, in which aberrations tend to remain roughly constant over 3-4 weeks. This raises the possibility that the ozone-induced abnormality is related to a postreplication repair process.

Carcinogenic or mutagenic effects of ozone have also been suggested by a number of other animal studies. Stokinger[199] noted that ozone exposure resulted in an increased incidence of pulmonary adenomas in a strain of mice prone to develop these benign neoplasms. However, the experimental findings were not reported in detail. A combination of ozone and gasoline has also been shown to cause an increased incidence of lung tumor in two strains of mice,[121,122] only one of which is prone to develop pulmonary adenomas.

A mutagenic effect of ozone on germ cells was suggested by the study of Brinkman et al.,[21] in which female mice exposed to ozone at 0.1-0.2 ppm 7 h/day 5 days/week for 3 weeks before birth, demonstrated a fourfold increase in neonatal mortality. A further report of this study noted increased blepharophimosis and jaw anomalies and a decrease in litter size.[213] An artificial oxidant smog mixture derived from irradiated auto exhaust has also been reported to cause an increase in neonatal mortality in mice.[109,125]

The toxicologic evidence indicating a possible role of ozone in human cancer may be summarized as follows:

- The biochemical mechanism of ozone toxicity appears to have many similarities with those of other agents, particularly ionizing radiation, that are known human carcinogens.
- In vitro studies with tissue-culture cell lines and bacteria have demonstrated ozone-induced chromosomal effects.
- Some animal studies with ozone or synthetic photochemical smog have shown chromosomal defects in circulating lymphocytes, a

more rapid appearance of benign pulmonary tumors, and an increase in neonatal mortality consistent with a mutagenic effect.

- One study of humans experimentally exposed to ozone showed transient development of minor chromosomal abnormalities in circulating lymphocytes.

The toxicologic studies must be considered in the perspective of an absence of epidemiologic evidence that links photochemical air pollution with human cancer. Despite the presence of significant concentrations of ozone in southern California for three decades, no associated increase in cancer has been observed. However, the relatively recent arrival and the peripatetic nature of Los Angelenos might obscure, for the present, a carcinogenic or mutagenic effect of ozone. Furthermore, it is necessary to consider the possible toxicologic interaction between ozone and other airborne carcinogens. The reported ozone-induced decrease in lung benzopyrene hydroxylase and cytochrome P-450 concentrations could be interpreted as protecting against aromatic hydrocarbon carcinogenesis in which *in vivo* activation appears necessary. Alternatively, it could be hypothesized that ozone would directly activate aromatic hydrocarbon carcinogens (e.g., by conversion to epoxides or other free-radical intermediates) and therefore promote the presumed carcinogenic process. This speculative discussion is presently solely to illustrate the potential complexities of the problem of ozone-induced carcinogenesis. Obviously, far more information is needed on the biochemical action of ozone and the interaction of ozone with other potential carcinogens. This would allow more confident assessment of the possible role of ozone in human carcinogenesis until definitive epidemiologic information is available.

EFFECTS ON REPRODUCTION

As noted previously, exposure of mice to simulated photochemical smog produced by irradiating diluted auto-exhaust mixtures resulted in reduced fertility and fecundity and decreased infant survival.[109] Lewis et al.[125] pursued this observation in greater depth and showed that the nonpregnancy rate was significantly ($p < 0.02$) greater (20 of 153) in female mice that were mated with male mice that had been exposed for 46 days to irradiated auto exhaust than in females that were mated with males that were in clean-air chambers (nine of 159). No differences in the groups with respect to the presence of copulatory plugs and absence of implantation scars in the nonpregnant females supported the authors conclusion that the differences in pregnancy rates were due to reduced fertility. In pregnant females that had been mated with irradiated-exhaust males, there were fewer uterine implantation scars and fewer pups per litter ($p < 0.05$) than in females mated with clean-air males. In addition, offspring viability was reduced in litters that were kept in irradiated-exhaust chambers. The concentration of oxidant (ozone) varied during each day of exposure, with peaks as high as 1.0 ppm. Daily nitrogen dioxide and carbon monoxide concentration peaks were about 1.5 ppm and 100 ppm, respectively. The results of these experiments suggested a possible mutagenic effect in male germ cells resulting from exposure to the oxidant mixture.

In an earlier study of a simpler oxidant smog mixture made by reacting ozone with gasoline vapors (oxidant concentration, 1.25 ppm in the mixture by neutral potassium iodide method), Kotin and Thomas[123] exposed male and

female mice to the synthetic oxidant mixture, to Los Angeles air, and to washed air. Significant differences from controls in reproduction effects were noted only in mating pairs in the synthetic smog mixture. These consisted of reduced conception rates, litter size, and survival of newborn (that were held in the exposure chambers until weaning). These authors also alluded to possible mutagenic effects associated with organic peroxides that would be expected in the synthetic mixture; however, they felt that their data on fecundity reflected an effect primarily on the females.

Brinkman et al.[21] and Veninga[213] exposed mated pairs of their laboratory's inbred gray mice and C57 black mice to ozone at 0.1 or 0.2 ppm 7 h/day 5 days/week for 3 weeks. There was little or not effect on litter size, compared with air-exposed controls; however, there was a greater incidence of neonatal mortality during the first 3 weeks of life in the litters of ozone-exposed parents. Offspring (C57 blacks exposed, in addition, for 3 weeks postnatally) from the 0.2-ppm-ozone groups had increased incidence of blepharophimosis and increased incidence of jaw abnormalities. The authors concluded that this was further evidence of a similar action of ozone and ionizing radiation (which produced the same effects at a dosage of 20 r/day).

These studies, although few, suggest that exposure to photochemical oxidants can influence fertility and fecundity in animals and that the general health of newborn animals is much more likely to be impaired by exposure to oxidants than that of their parents. Whether the changes observed in reproduction variables can be related to mutagenic actions of

ozone, discussed earlier, remains to be determined. In any event, it seems logical that effects of low concentrations of ozone and other photochemical oxidants on reproduction must be indirect and may be mediated by endocrine or ozone-biologic reaction products.

CENTRAL NERVOUS SYSTEM AND BEHAVIORAL EFFECTS

It is somewhat surprising that possible central nervous system (CNS) or behavioral responses to ozone exposure have not been subjected to greater investigation, in view of the reports of headache and drowsiness in humans exposed to ozone and the apparent CNS depression that occurs as an early sign of ozone interaction in animals.[200,201] Xintaras et al.,[221] in an application of the evoked-response technique, found a depression in the specific visual cortex and in the superior colliculus of rats exposed to ozone at 0.5-1.0 ppm for 1 h. However, Eglite[53] found no change in rat behavior or chronaxial muscle ratios after 93 days of exposure to ozone at 0.6 ppm. Lagerwerff[124] demonstrated in human volunteers that repeated exposure to ozone at concentrations of 0.02-0.05 ppm resulted in psychophysiologic or sensoriphysiologic changes, including decreased visual acuity, increased peripheral vision, altered extraocular muscle balance, lethargy, and difficulty in concentrating.

As mentioned previously, decreased voluntary running activity of mice was one of the most sensitive indexes of an effect of ambient photochemical oxidant air pollution[55] or simulated oxidant smog that was produced in the laboratory by irradiating diluted auto-exhaust gasses.[109,147] Whether this effect of the mixtures was due to only ozone or other oxidant is not certain. It is plausible to conclude that ozone was the effective

agent, in view of the fact that Murphy et al.[153] showed that ozone at as low as 0.20 ppm (during a single 6-h exposure) reduced voluntary running activity of mice by 50%. The 6-h exposure used in those studies coincided with the maximal activity period of the animals (i.e., darkness, in adapted mice), so it seems that the effect of ozone overcame strong natural motivation for running activity. Furthermore, Konigsberg and Bochman[120] noted a concentration-related reduction in the gross motor activity of rats exposed to ozone at 0.1-1.0 ppm. As reported for oxidant mixtures,[109] mice exposed continuously to ozone at 0.3 ppm adapted and returned to normal activity in about a week.[153] However, the same mice were still susceptible to the activity-reducing effect of higher concentrations (0.6-0.8 ppm), and adaptation to control activity did not occur for at least 7 days of continuous exposure.[153] Fletcher and Tapell[68] reported that the voluntary running activity of rats was reduced by 84% during continuous exposure to ozone at 1 ppm for a week. Boche and Quilligan[18] also demonstrated that the oxidant mixture produced by ozonizing gasoline vapors (ozone content, 0.4 ppm or greater) caused a decrease in running activity in mice.

These studies, although demonstrating that reduction of voluntary running activity in mice is a very sensitive biologic indicator of oxidant and ozone, did not reveal the mechanism of the effect. It is possible that this effect represents only a precautionary reaction of the organism to avoid moving about when its senses detect (by odor or irritation) a strange and unpleasant stimulus. This would be consistent with the observation of avoidance, by mice, of a cage ventilated with ozone at

0.6-1.1 ppm in preference for a clean-air cage.[172] Reduction of voluntary running activity might also be secondary to lung irritation or inflammation; however, the concentrations for activity reduction are below those generally associated with edema or other signs of local injury in the lung. Finally, one could evoke a systemic mechanism of action as a direct effect of circulating ozone (which seems unlikely) or of a CNS-depressant oxidant-biologic reaction product formed locally in the lung. Further studies would be required to determine whether these or other mechanisms are involved.

SUMMARY OF DOSE-RESPONSE RELATIONSHIPS

The acute lethal action of ozone is due to its capacity to produce pulmonary edema. The LC_{50} for rats and mice exposed for a single 4-h period is approximately 6 ppm, and cats, rabbits, guinea pigs, and dogs (in that order) are decreasingly susceptible to the lethal action.[199] Numerous reviews have considered the toxicity of ozone at lethal and lower concentrations.[113,146,162,199-202,212]

In the foregoing discussion and in Table 8-2, attention has focused on studies in laboratory animals that have been exposed to ozone concentrations of about 1 ppm or less, because results of studies conducted with such concentrations are thought to be more directly relevant to ambient oxidant air pollution, which is the source of exposure for large human populations. In Table 8-2, no attempt has been made to list all studies; more comprehensive summaries can be found in some of the monographs cited. Instead, the table cites studies thought to be most useful for evaluating the health implications of exposure to low concentrations

of ozone, and the concentrations listed are the lowest at which the described effects have been observed. Unfortunately, many studies have not included a sufficient range of experimental concentrations to permit construction of reliable dose-response curves or to determine what, if any, would be a "no-observed-effect" concentration for the experimental conditions used. Although it is understandable that research scientists find little stimulation in conducting exposure experiments at concentrations that fail to produce changes in the biologic system in which they are interested, the conduct (and reporting) of such experiments is extremely important for a pragmatic evaluation of the implications of positive findings for human health.

The table illustrates the wide variety of biologic effects produced in laboratory animals exposed to relatively low concentrations of ozone. Obviously, some effects have more serious health implications than others.

Table 8-2

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal	Reference
0.08	3 h	Increased mortality from pulmonary infection with Streptococcus	Mouse	36
0.1	7 h/day, 5 days/wk for 3 wk	Increased incidence of neonatal mortality in litters of exposed parents (related effects and reduced fertility noted in synthetic oxidant smog)	Mouse	21,109,123,125,213
	7 days	Increased succinate-dependent lung mitochondrial oxygen consumption in rats on diet relatively low in vitamin E; no significant effect in vitamin E-replete rats at this concentration of ozone	Rat	159
0.2	3 h	Degenerative changes in Type I alveolar cells; later replaced by Type II cells	Rat	197
	Continuous for 28-32 days	16% increase in lung volumes; overdistention at high lung volumes, suggesting some change in elasticity; no change in respiratory frequency, tail length, or external appearance	Young rat	10
	6 h or continuous for 7 days	Decreased voluntary runway activity during exposure; no-effect concentration not reported; reduced gross motor activity	Mouse	153
	7 h/day, 5 days/wk for 3 wk	Increased incidence of blepharophimosis and jaw abnormalities in neonates	Mouse	213
	7 days	Increased succinate-dependent lung mitochondrial oxygen consumption	Rat	157

Table 8-2 (Cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal	Reference
0.2 (Cont.)	Continuous for 8 days	Dose-related increased activity of lung glutathione peroxidase and glutathione reductase (glucose-6-phosphate dehydrogenase activity significantly increased at 0.5 ppm)	Rat	31
	5 h	Lymphocyte chromosomal breaks	Hamster	223,224
0.2-0.25	0.5-2 h	Increased red-cell spherocytosis after *in vitro* radiation	Mouse, rat, rabbit, man	21
0.25	4-6 h	Morphologic changes in medium-sized airways	Cat	16
0.25-0.5	6 h	Threshold for lung edema formation with ^{131}I albumin test	Rat	5
	3 h	Decreased lysozyme, acid phosphatase, and β-glucuronidase activity in alveolar macrophages (appears to be linearly related to dose up to 1 ppm)	Rabbit	2,111
0.26-0.5	4.6 h	Increased lung flow resistance in 2 animals at 0.26 ppm; effect in all at 0.5 ppm	Cat	215
0.34	2 h	30% increase in frequency of breathing; 20% decrease in tidal volume	Guinea pig	153
0.37-0.5	2 h	Decreased red-cell acetylcholinesterase and increased osmotic fragility (no effect at 0.25 ppm)	Man	99
0.4	6 h/day, 5 days/wk for 10 months	Increased serum trypsin protein esterase	Rabbit	169
0.4-0.7	4 h	Conjugated diene bonds, suggesting lung lipid peroxidation	Mouse	90

Table 8-2 (Cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal	Reference
0.5	6-10 h	Minor chromosomal abnormalities	Man	138
	3 h	Inhibition of intracellular hydrolytic enzymes of alveolar macrophages; increased fraction of polymorphonuclear leukocytes	Rabbit	3
	165 min	Alterations in blood, including red-cell membrane and enzyme changes and increased serum vitamin E and lipid peroxides	Man	23
	6 h	Decreased lung DNA synthesis	Mouse	56
0.5-1	1 h	Decreased electric response of specific areas of brain with evoked-response technique	Rat	221
0.54-0.88	Continuous for up to 3 wk	Morphologic changes in distal and respiratory bronchioles, alveolar ducts, and associated alveoli	Young rat	74
0.6	Not applic.	Avoidance of cage ventilated with ozone	Mouse	172
	93 days	No change in behavior or chronaxial muscle ratios	Rat	53
0.68	4 h	Decreased rate of bacterial killing in lungs *in vivo*	Mouse	95
	2 h	No significant increase in respiratory flow resistance	Guinea pig	153
0.7-0.8	Continuous for 7 days	Increased acid phosphatase in specific lung areas determined histochemically	Rat	29

Table 8-2 (Cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal	Reference
0.7-0.8 (Cont.)	Continuous for 5-7 days	Increased activity of lysosomal hydrolases in whole-lung homogenates	Rat	48
0.75	3 h	Decreased benzopyrene hydroxylase in lung and tracheobronchial mucosa	Hamster	166
	4-8 h	Histologic changes in parathyroid glands	Rat	8
0.8	Continuous for 7 days	Histochemically determined alteration in several lung enzyme activities	Rat	30
	Continuous for 8 days	Increased activity of lung and plasma lysozyme (no effect at 0.2 and 0.5 ppm)	Rat	31
	7 days	Increased activity of lung pentose shunt and glycolytic enzymes; decreased lactic dehydrogenase	Rat	32
0.84	4 h/day, 5 days/wk for 2 wk	Increased susceptibility to respiratory infection with Klebsiella pneumoniae	Mouse, hamster	139
0.85	4 h	Heinz bodies in circulating red cells; further exposure led to decrease in Heinz body formation	Mouse	136,137
0.9	Continuous	Lungs 38% heavier than those of normals; 50% dead in 3 wk	Young rat	74
1	1 h	Chemical changes in ground substance and lung protein	Rabbit	26
	4 h	Engorged blood vessels and excess leukocytes in lung capillaries	Mouse	191

Table 8-2 (Cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal	Reference
1 (Cont.)	90 min	Decreased lung cytochrome P-450	Rabbit	93
	1 h	Formation of carbonyl compounds and alterations of hyaluronic acid in lung	Rabbit	26
	8-24 h/day for 18 months	Alteration in catechol-O-methyltransferase and monoamine oxidase of brain tissue	Dog	211
	3 h/day for 2-3 successive days	Prolongation of phenobarbital sleeping time (no effect after 1 or 4-7 days)	Mouse	76
	Continuous for up to 18 months	Bronchitis; bronchiolitis; emphysematous and fibrotic changes; acceleration of lung-tumor development	Mouse	199
	Continuous for 1 wk	Decreased voluntary running activity	Rat	68
1-3	Continuous for 18 months	Thickening of bronchioles and respiratory bronchioles; barely noticeable at 1 ppm; at 3 ppm, formation of peribronchiolar collars with resulting narrowing of small airways	Dog	75
1.08	2 h	47% increase in respiratory flow resistance	Guinea pig	153
1.3	3 h	Increased susceptibility to Klebsiella pneumoniae	Mouse, hamster	139

[a] Concentrations listed are lowest for which observed effects have been reported.

RECOMMENDATIONS FOR RESEARCH

Enhanced susceptibility to respiratory exposure to infectious agents is of considerable potential public health significance. This has been reported to occur in mice exposed to ozone at as low as 0.08 ppm, the lowest reported "effect" concentration in laboratory animal studies. It seems essential, therefore, to continue and expand research on the effect of ozone and other photochemical oxidants on physiologic protective mechanisms of the lung. Appropriate dose-response studies should be included to confirm or establish the exposure concentration-time relationships that result in increased susceptibility to inhaled microorganisms, and studies of the cellular responses and mechanisms should be conducted with a view to providing methods that are applicable to epidemiologic studies in oxidant-exposed human populations.

Research designed to elucidate the pathophysiologic implications of reversible changes in lung function, histology, and biochemistry that have been observed at concentrations of 0.2-0.5 ppm would be especially valuable in evaluating the significance of these changes for health. In particular, it would be useful to determine what, if any, causal or correlative relationships exist between different effects detected by various experimental assay systems and between reversible changes and more chronic effects, such as reduced lung elasticity, fibrosis, and adenoma formation. Studies on laboratory animals are particularly suited to this type of mechanism-correlative research; but, with appropriate experimental designs, such research should be very useful in determining which methods or biologic changes can be usefully applied to clinical or

epidemiologic studies. At the same time, such research could determine whether some of the changes (e.g., increased activity of enzymes involved in cell redox systems) are indicative of injury or are homeostatic adaptive responses. This information will have value in predicting potential injury in the "average" population and possible help to identify people who are hypersusceptible by virtue of some deficiency in adaptive protective mechanisms. Related to these research efforts, further investigation of the influence of oxidant exposure on enzymes that biotransform other inhaled chemicals (e.g., aromatic hydrocarbons) and the interaction effect of dietary antioxidants (e.g., vitamin E) should be pursued, particularly in relation to oxidant-induced oxidation of membrane lipids and free-radical formation.

There are several scattered reports of extrapulmonary effects of ozone exposure in laboratory animals. Some (e.g., chromasomal aberrations in hamster lymphocytes) occur at or near concentrations that cause local effects in the lung and portend serious and long-term health implications. Others(e.g., reduction of voluntary activity) may be transient or reversible, but nevertheless contribute to decrements in performance or well-being. It is particularly important that further research be conducted to confirm (or refute) these reported extrapulmonary actions of ozone and the exposure concentrations at which they occur. If confirmation of their occurrence is obtained at or below the currently reported effective exposure concentrations, useful research efforts could be directed toward determining whether they are direct or secondary effects and toward identifying the chemical species responsible. More

importantly, confirmation of an isolated effect on cell genetic material would demand a thorough expert evaluation of its significance on the basis of existing knowledge of the long-term implication of the effect and, accordingly, intensified laboratory and epidemiologic research.

DISCUSSION

During the last decade, toxicologic research on the effects of ozone in laboratory animals has demonstrated that exposure for a few hours to airborne concentrations of less than 1 ppm produces numerous changes in cell and organ structure and function. The limiting concentrations required to produce these changes appear to differ somewhat among different species of laboratory animals and with the particular type of effect under investigation. However, there seems to be little doubt that several functional and morphologic indexes of response to ozone are altered with exposures to ozone concentrations of about 0.2-0.5 ppm. Many of the functional and morphologic changes produced in experimental animals exposed to ozone as a single contaminant gas have also been demonstrated to occur with exposure to complex mixtures of laboratory-produced or ambient photochemical oxidant air pollution.

Most research has focused on the effects of ozone on lung function, morphology, and biochemistry. Altered lung function, observed in studies of the mechanical behavior of lungs of several species, has been noted as the result of a few hours of exposure to ozone at 0.2-0.3 ppm. Improved techniques for detecting minimal pulmonary edema and for examining the microstructure of cells of the respiratory tract indicate that structural alterations also occur with short exposures to this range of concentrations.

Furthermore, altered activities of various lung enzymes or enzyme systems, particularly those involved in defending against oxidant stress, have been noted by several investigators to occur at concentrations of 0.2 ppm. Although all these effects appear to be accentuated by higher concentrations of ozone, indicating a dose-response relationship, most investigations have not determined (or have failed to report) whether lower concentrations fail to produce such changes. In spite of the fact that biochemical, functional, and morphologic effects have all been detected at about the same low exposure concentrations, there has been little systematic investigation to determine whether these effects are causally related. Some effects noted in these experiments might be considered adaptive, rather than injurious. For example, the increased activities of lung glutathione peroxidase and glutathione reductase that have been demonstrated after exposure to 0.2 ppm may represent an adaptation or compensation to an injurious effect, such as lipid peroxidation, which, although detected only at exposures to 0.4 ppm and above, may go undetected at lower exposure concentrations because of the insensitivity of current assay methods. In spite of these unknowns, it is noteworthy that, in the one case in which it is possible to make direct comparisons of the effects of ozone in laboratory animals and in humans in controlled experiments—i.e., lung-function studies—the minimally effective concentrations are nearly identical, around 0.3 ppm. It is tempting, therefore, to speculate that humans might also sustain the biochemical and morphologic changes observed in laboratory animals exposed to this concentration; but such a conclusion must await mechanistic information concerning the relationships

between the different types of effects or independent demonstration of these effects in man.

Recent studies involving repeated or prolonged exposures of laboratory animals to ozone suggest that changes that are indicative of chronic lung diseases (such as decreased elasticity of the lungs, hypertrophy of bronchiolar epithelium, and deposition of connective tissue) also require concentrations of 0.2-0.5 ppm. At slightly higher concentrations, fibrotic changes in the lungs have been observed histologically; this is consistent with a reported increased rate of collagen synthesis in the lung. It appears, therefore, that the changes observed with short-duration exposures, although generally reversible on cessation of exposure, are as sensitive in evaluating the injury potential of exposure to ozone as are long-term exposure studies. There have been, however, very few truly chronic exposure studies (i.e., covering a major part of the lifetime of the species) on which to base conclusions.

Another subject of active investigation concerning the effects of ozone is related to the mechanisms of defense against inhaled microorganisms. It has been shown that exposure of a few hours results in a marked increase in the susceptibility of animals to controlled doses of infectious organisms introduced into the lung. In fact, this criterion of an effect is the most sensitive of any that have been reported, with significantly increased susceptibility of mice to one microorganism occurring after exposure to an ozone concentration as low as 0.08 ppm. Other reports referring to different microorganisms or different species suggest that somewhat higher concentrations are required, but clearly this effect is noted at less than 1.0 ppm. Research in the mechanism of

ozone's effectiveness in decreasing resistance to infectious agents suggests an action on the numbers and viability of lung macrophages. Aside from the obvious practical implication of these findings for carefully planned epidemiologic studies on the incidence of lung infection in human populations exposed to oxidant air pollution, they have stimulated more basic research in the physiology and biochemistry of lung macrophages that may have implications beyond the question of oxidant toxicity.

Earlier toxicity studies had suggested that the hazard from repeated ozone exposure might be reduced, inasmuch as animals became tolerant to the acute pulmonary edematogenic action of ozone. However, more recent studies, which demonstrate that tolerance to the ordinarily ozone-induced increase in susceptibility to infectious microorganisms or to the effects on respiratory mechanics does not develop, suggest that the tolerance phenomenon would have little protective value with respect to repeated exposure to ambient oxidant smog.

There are several reports of noteworthy extrapulmonary effects in laboratory animals with concentrations of about 0.2 ppm. These include reduced voluntary activity, chromosomal aberrations in circulating lymphocytes of hamsters, increased neonatal mortality, and greater incidence of jaw abnormalities in offspring of ozone-exposed mice. The mechanisms of these reported effects and whether they are due to direct actions of absorbed ozone, some secondary reaction product, or secondary responses to the stress of local actions in the lung are largely unknown. However, reported analogous effects in humans exposed to ozone, such as

changes in visual acuity and headache (possibly related to the reduced activity in animals) and minor chromosomal abnormalities in circulating lymphocytes, require that these extrapulmonary effects be considered in the evaluation of the hazard of ozone exposure. Furthermore, the chromosomal aberrations in hamsters and the reports of mutagenic activity of ozone in a variety of microorganisms, tissue cultures, plants, and insects raise the questions of the possibility of a genetic or carcinogenic hazard of this gas. Indeed, earlier studies at higher concentrations of ozone or mixtures of ozone with hydrocarbons have suggested a tumorigenic action in the lungs of mice. Although present evidence does not justify a definitive conclusion that exposure to ozone implies a mutagenic or carcinogenic hazard, prudence requires that this possibility should not be dismissed until it has been definitively tested experimentally and epidemiologically.

The possibility of interactions of ozone with other environmental stresses has received relatively little recent attention. Two exceptions are noteworthy, however: reports of increased susceptibility to some of the actions of ozone in animals that are deficient in vitamin E or the converse (protection conferred by administration of vitamin E) and observations that exposure to ozone at less than 1.0 ppm reduces activity of the cytochrome P-450 mixed-function oxidase activity of lungs. Although the implications of these observations are not yet clear, they remind us that nutritional variations or exposure to foreign chemicals that are metabolized by lung mixed-function oxidases may provide bases for unanticipated qualitative or quantitative effects associated with oxidant exposures.

On the basis of the foregoing discussion, it appears that, if traditional criteria for hazard evaluation are applied to the toxicologic data on experimental animals, there is little room for complacency regarding current ambient concentrations of ozone. Functional, biochemical, and structural effects in both pulmonary and extrapulmonary systems have been reported by numerous investigators at or near concentrations that are at least occasionally achieved in some polluted urban centers. Unfortunately, there are no adequate methods for extrapolating data to obtain reliable quantitative estimates of population risk at environmental concentrations near the standard, and there is no assurance that the risk is zero.

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CHAPTER 9

CONTROLLED STUDIES ON HUMANS

Most standards for safe concentrations of oxidants and other photochemical pollutants are based on information about their effect on human health.[1] Such information is acquired through the interplay of different fields of scientific endeavor, including epidemiologic investigation of pollutant-exposed human groups and controlled experimental study of animals or volunteer human subjects. Epidemiologic studies of humans can be used to obtain dose-response information on polluted ambient air, but they are limited by cost factors, dose-range availability, the presence of interfering pollutant substances, and the problems caused by the presence of many uncontrolled variables. Most controlled experimental investigations have been conducted with animals. However, there is no rigorous way of extrapolating animal dose-response data to humans. There is a need, then, for comprehensive human experimental studies that are carefully controlled and documented to ensure reproducibility and to withstand challenge by proponents of other standards of safe concentrations. Such studies can establish the presence and importance of acute health responses in normal and hyperreactive people. Dose-response information, including minimal-measurable-effects concentrations, can be determined. Hypotheses can be developed and then subjected to animal or epidemiologic investigation. Typically, controlled human studies on the health effects of specific pollutants use fixed concentrations, an absence of interfering pollutants, controlled environmental conditions, and well-characterized volunteer subjects. Risk is inherent in any research involving humans, and it is axiomatic that reasonable and proper care should be taken.[36a] Prudence in the design of

controlled human experiments requires that, when available, information from animal and epidemiologic work be used to delineate ways in which critical human studies are likely to be most useful.

The purposes of this chapter are to review available test methods and protocol designs for controlled human studies, to review and discuss published data, and to make recommendations for future studies.

EXPERIMENTAL METHODS

The basic design of studies on effects of pollutants should seek to maximize information relevant to public health. Tests must be reliable and sensitive, the experimental air environment must be rigorously controlled, and the manner in which subjects are exposed to this environment must simulate ambient exposure. These constraints impose complications and necessitate a focus on environmental control and monitoring, physiologic testing, and evaluation of symptoms and clinical observations.

Controlled-Environment Chambers

Construction and Monitoring Equipment. A survey of the environmental control and monitoring technology used in several experimental studies[1,7,8,9,32] indicated significant limitations in experimental control capability. There are seven controlled-environment chambers or clean-room facilities in the United States for human exposure (community air pollution inhalation) from which studies have been reported. Another is under construction at the University of North Carolina in association with the EPA at Chapel Hill. There are three chambers in Canada of similar design.

Table 9-1 lists design features of the exposure chambers in the United States that have air-cleaning equipment. The facility at the

TABLE 9-1

Controlled-Environment Chambers or Clean-Room Facilities for Community Air Pollution Short- Or Long-Term Human Exposure, United States[a] Facility[b]

Item or Condition	H.S.C.	U. Md.	U. Pa.	R.L.A.	St. V	St. B[c]	N.Y.U.
Temperature Control	+	+	+	+	+	+	+
Humidity Control	+	+	+	+	+	+	+
Pressure Control	+	-	+	-	-	-	-
Sound Control	+	-	+	+	-	+	-
High-Efficiency Particle Filter	+	+	+	+	+	+	+
Laminar Flow[d]	-	+	-	[e]	-	+	-
Charcoal Filter	+	+	+	+	+	+	+
Catalytic Filter[f]	+	-	-	+	-	-	-
Scrubber[g]	+	-	-	-	-	-	-
Purafil filter[h]	-	-	-	+	-	-	-
Active[i]	-	+	-	+	-	+	-

[a] Based in part on information from an EPA - sponsored workshop on controlled-environment-chambers, April 26-27, 1971, Research Triangle Park, N.C.

[b]
H.S.C. = Hospital for Sick Children, Washington, D.C.
U. Md. = University of Maryland Hospital, Baltimore.
U. Pa. = University of Pennsylvania Hospital, Philadelphia.
R.L.A. = Rancho Los Amigos Hospital, Downey, Ca.
St. V. = St. Vincent's Hospital, New York, N.Y.
St. B. = University of California, Santa Barbara.
N.Y.U. = New York University, New York, N.Y. (Clean-room facility has been dismantled).

[c] More than one chamber available. This describes the plexiglass chamber used for ozone and peroxyacetylnitrate studies.

[d] Refers to use of an intake plenum to ensure fairly uniform air flow and negligible gas concentration gradients within the region of the exposure chamber occupied by subjects.

[e] Can be operated with intake flow either through or bypassing the plenum.

[f] Heated or nonheated bed designed to oxidize carbon nonoxide and light hydrocarbons (not removed by activated charcoal) to carbon dioxide and water.

g The liquid acid gas scrubber is packed with polypropylene and uses sodium carbonate solution.

h Aluminum oxide pellets impregnated with potassium permanganate.

i As judged by personal communication or recent publication.

University of Maryland Hospital, Baltimore,[37] has a chamber with activated-charcoal and high-efficiency particle filters and controlled temperature and humidity. St. Vincent's Hospital and New York University, New York City, each have a clean-room facility. The University of Pennsylvania Hospital, Philadelphia,[25] has a self-contained, reinforced-concrete climate-controlled chamber, with pressure controls from 28 to 32 in. Hg (about 710 to 810 mm Hg, or 95 to 108 kilopascals). Rancho Los Amigos Hospital, Downey, California, has a totally enclosed steel chamber. Inlet air is heated and passed through a Hopcalite catalyst bed, filtered by high-efficiency particle filters, activated charcoal, Purafil (aluminum oxide pellets impregnated with potassium permanganate), and a Mine Safety Appliance catalytic adsorbent bed.[28a] The controlled-environment facility at the Hospital for Sick Children, Washington, D.C., is a self-contained, reinforced-concrete structure that can provide clean or specifically polluted air of monitored composition along with controlled temperature, pressure, and humidity. The living area for the subjects is a two-room apartment with bath and minimal kitchen facilities (600 ft^2, or 56 m^2).[53] The University of California at Santa Barbara has two chambers for temperature - and humidity-controlled environmental studies. A third chamber is used for altitude simulation, and a fourth for ozone and peroxyacetylnitrate (PAN) studies. The latter chamber is 6.5 ft (2m) wide, 10 ft (3m) long, and 19 ft (5.8m) high and is constructed of plexiglass. A chamber complex at the University of Wisconsin in Madison is designed primarily for studies of occupational inhalation toxicology.[55]

A description of the environmental-control chamber for human experimental studies at McGill University, Montreal, exemplifies the other two Canadian facilities -- at the Gage Research Institute, Toronto, and

McMaster University, Hamilton, Ontario. It is a transparent chamber, 12 ft (3.7 m) long, 8 ft (2.4 m) wide, and 6 ft (1.8 m) high constructed of 14 6x4-ft (1.8 x 1.2-m) panels of plexiglass held together with angled metal. The chamber is built within an ordinary air-conditioned working laboratory, with a door in the middle of one of the 12-ft (3.7-m) sides. Near the bottom of the chamber a hole has been cut to accommodate a 9-in. window fan, which gives the desired incoming atmosphere. Air from the laboratory is not purified before entering the chamber. At the end of the chamber opposite the fan, two outlet pipes, 6.5 and 3.75 in. (16.5 and 9.5 cm) in diameter, run from the chamber ceiling to a fume hood. Air passes through the chamber at approximately 12,000 ft^3/h (336 m^3/h), resulting in about 20 volume changes hourly. The temperature and humidity controls are those for the building in which the chamber is housed.

For the most reliable results, chamber environment should be monitored continuously with instruments and techniques equivalent to those used in ambient-air monitoring networks. Calibration of instruments should follow recommendations by appropriate agencies and be checked by cross comparisons with those in other analytic laboratories.

Ideally, two monitoring instruments, each operating on a different principle, should be used for each gaseous pollutant under study. Ozone and nitrogen oxides can be monitored with chemiluminescence analyzers, which provide fast response and freedom from interference by other pollutants. Total oxidants can also be monitored by the neutral potassium iodide solution and Saltzman reagent methods with continuous-flow colorimetric analyzers. A nondispersive infrared analyzer and an oxidative electrochemical analyzer

can be used for monitoring carbon monoxide, and light-scattering counters for monitoring particles.

Environmental Variables. In human experimental studies, variables to be considered and controlled include pollutant gas concentration, humidity, temperature, light intensity, noise, particles, and the presence of other gases.

The relative humidity, air temperature, noise, and artificial lighting must be controlled in environmental exposure chambers, because they can influence a subject's response directly or indirectly. At high temperature, heat stress can dominate the symptomatic and physiologic responses of human subjects. The rates of some chemical reactions of exposure gases depend heavily on temperature, so temperature should be controlled. This can be done with refrigerated cooling coils in the air-conditioning section of air supply systems, heaters, and thermocouple sensors. Extremes of relative humidity are known to cause discomfort in humans. The equilibrium size of hygroscopic aerosol particles, the degree of conversion of sulfur dioxide to sulfurous acid in sodium chloride droplets,[41] and the extent and rate of chemical reaction of mixtures of such pollutant gases as sulfur dioxide, ozone, and ammonia depend on the relative humidity of the chamber air. Cooling coils in air-conditioning units, and the injection of purified steam can be used to regulate relative humidity. Artificial lighting within human-exposure chambers should be designed to ensure proper illumination, but to avoid potential experimental complications. If the intensity is too high, the air may overheat. Ultraviolet irradiation should be avoided to prevent photochemical reactions from forming uncontrolled and unknown reaction

products in the chamber. If ultraviolet irradiation of gases is desired to form specific oxidant compounds for human exposures, it should be carried out in separate chambers to avoid contamination of the human-exposure chamber with intermediate products and to prevent the subjects from being directly exposed to harmful radiation. Conventional tungsten and fluorescent lighting meet the illumination requirements of human-exposure chambers.

High levels of noise known to cause discomfort to humans are unacceptable. Moderate noise also becomes undesirable in some experimental situations, if it is distracting or interferes with speech communication.

During controlled exposures of human subjects to specific compounds like ozone, the concentrations of suspended particles and trace gases must be known and minimized to ensure that health effects can be attributed solely to ozone. The air purification units for the environmental chambers are designed to remove most of the particles and pollutant gases from the ambient air. Prefilters and efficient absolute filters are used to remove 99% of the particles with diameters of 0.3 μm or more. Catalytic beds, adsorption beds, and activated-carbon and chemical filters are used to convert carbon monoxide to carbon dioxide and to remove most of the other gaseous hydrocarbons, sulfur dioxide, nitrogen dioxide, nitric oxide, and ammonia. Condensation nuclei counters, which are commercially available, can be used to monitor the total number of particles with diameters greater than 20A. Light-scattering nephelometers can approximate the total number concentration of particles with diameters of 0.1 - 1 μm. Single-particle optical counters with multichannel analyzers can be used to monitor the number of particles in finite size intervals between 0.2 and 10 μm. Alternatively, cascade impactors and particle filters can be used to monitor the total mass

concentration and the size distribution of the aerosol in the chamber. Gas monitors are commercially available for sulfur dioxide, nitrogen dioxide, nitric oxide, carbon monoxide, and ozone. Ammonia and these other gases can also be collected by bubblers and analyzed by wet chemical methods if proper precautions are taken to account for interferences. Trace hydrocarbons can be monitored by taking bag samples and analyzing them by gas chromatography, mass spectrometry, or infrared spectrophotometry.

In the design of controlled exposures to gases and particles, separately or in combination, extreme care is needed to avoid formation of interfering species. For example, in a study of the effect of a mixture of nitrogen dioxide and ferric oxide aerosol in dogs,[39] significant quantities of ammonium and nitrate ions were found in particles filtered from the air of the exposure chamber. Ammonia and trace hydrocarbon gases also emanate from sedentary and exercising humans. To prevent these contaminant gases from building up in the chamber, a continuous flow of fresh air through the chamber must be maintained.

In exposures of humans to artificially generated aerosols, where the information is to be relevant to ambient aerosols, several factors are important: the particle diameter distribution must be fairly constant and fall within size ranges typical for the given compound in the ambient air, the chemical composition of the aerosol must be stable and predictable, and the electric charge distribution of the aerosol must simulate that of normal atmospheric aerosols.

Selection and Characterization of Subjects

A minimal group size is difficult to specify. Studies on similar subjects could be expected to give the least variability in results; thus,

dose-response information on relatively small groups of medically characterized subjects could provide a basis for extrapolating to essentially similar population groups. Useful results have been obtained with as few as eight subjects.[31] Healthy, young males probably should be studied initially because some variations are known to accompany aging and some problems are peculiar to females (e.g., the estrus cycle and pregnancy). Later studies of older males and females will, however, be required for complete information.

Volunteers with disease who can give informed consent and for whom some expected benefit balances the expected risk can be considered as potential subjects. If those selected for the study are smokers, this information and an estimate of the degree of smoking are needed, because systematic differences between smokers and nonsmokers have been found.[32,38]

People with known or suspected cardiopulmonary disease who are referred by a physician for diagnostic environmental stress-testing may also serve as subjects. Testing under environmental stress of persons who are suspected hyperreactors to pollutants is analogous in concept to exercise stress-testing of suspected cardiac patients. However, in contrast with the general availability of exercise stress-testing laboratories, the expense involved in an operational facility for environmental stress-testing limits its availability to research centers. Accordingly, a person who is referred for clinical testing by his physician may well become part of investigative programs and be asked to volunteer for additional procedures.

In selection of subjects, ethical considerations are dominant and limit the deliberate exposure of minors or of others who are unable legally to be volunteer subjects.

Among the criteria for selecting subjects with close similarities, socioeconomic status cannot be ignored any more than nutritional status or genetic background. Finally, place of residence and prior occupational pollution exposure may be important.

Measurement of Human Response

In addition to the visual monitoring of subjects during testing, clinical assessment can be accomplished by having the project physician interview each subject concerning symptoms, with a standard questionnaire immediately after exposure. Also, subjects can keep a record, on a standard form, of symptoms experienced during and after exposure. It is well to note here that caution should be exercised in interpreting symptoms, because these are unlikely to be "blind" studies.

Much progress has been made in recent years in the measurement and understanding of lung physiology. Usually, several methods are available for the measurement of each characteristic of lung function of interest. Comprehensive descriptions of the various pulmonary function tests are readily available,[6,12,20,29] so a complete review will not be attempted here.

Because the respiratory tract is an initial target of any air pollutant challenge, it usually receives primary attention in tests to determine irritant effects of exposure. Other aspects of interest include hematology, blood enzyme biochemistry, eye irritation, and psychomotor performance. Constriction of the large airways, maldistribution of ventilation due to narrowing in some small airways, constriction of peripheral lung units, and mechanical or gas diffusion impairment due to edema

are possible effects of insult by pollutants. A variety of pulmonary tests are required to examine the possibilities.

Flow-volume curves:[13] The characteristics measured by the maximal expiration are forced vital capacity (FVC), 1-s forced expiratory volume (FEV_1), peak expiratory flow rate ($\dot{V}max$), and flow rates at 50% and 25% of the remaining FVC ($\dot{V}_{50}$, $\dot{V}_{25}$) for partial and maximal flow-volume curves. These measurements give an easily obtained, relatively reproducible evaluation of overall pulmonary mechanical performance, but provide little information on the mechanisms responsible for any observed changes.

Airway resistance (R_{aw}) and thoracic gas volume (TGV):[22,23] The measurement of R_{aw} is probably more sensitive than maximal flow measurements for constriction of large airways, but it is also more difficult to perform and is less stable. These problems similarly affect the measurement of TGV, which, however, may be useful for detecting gas trapped as a consequence of airway dysfunction (in combination with a gas-dilution lung-volume determination).

Total respiratory resistance(R_t):[24,26,29] Resistance can be measured at pressure perturbation frequencies of 3, 6, 9, and 12 Hz. Unfortunately, this measurement is affected by changes in upper-airway configuration, which may complicate the detection of changes in pulmonary airways per se. As predicted by Otis,[43] the method is believed capable of detecting asynchronous mechanical behavior (unequal regional ventilatory time constants), which otherwise can be documented only by the considerably more difficult measurement of dynamic lung compliance.

Closing volume (CV):[3,16] Buist and Ross[16] measured the lung volume at which closure of a significant number of small airways presumably occurs,

as well as estimating of residual volume (RV) and total lung capacity (TLC) through the expired nitrogen concentration. In another study,[17] they presented a method to estimate the uniformity of ventilation distribution by measuring the slope of the alveolar nitrogen plateau.

Static lung compliance (C_{st}) and dynamic lung compliance (C_{dyn})[40] are measured from recordings of transpulmonary pressure and respiratory flow and volume. Dynamic compliance in the tidal range is measured in a series of breaths each at normal frequency and at other frequencies -- such as 20, 40, 60, 80, and 100 breaths/min -- with total volume monitored and kept constant. Static compliance is measured by interrupting airflow intermittently during an inspiration from functional residual capacity (FRC) to TLC, followed by an expiration to RV. Each determination is preceded by an inspiration to TLC to give a consistent volume history. Compliance measurements are indispensable for documentation of changes in the mechanical characteristics of the lung, particularly the development of unequal time constants. Unfortunately, the measurements are somewhat unstable and require considerable effort on the part of subjects and investigators. In some studies, these tests are performed only on a subgroup of subjects selected for motivation and performance.

Pulmonary diffusing capacity ($D_{L_{CO}}$) can be determined by the single-breath carbon monoxide method according to a technique developed by Ogilvie *et al.*[42] For example, a test gas containing 0.15% carbon monoxide and 10% helium in air can be used. In calculating $D_{L_{CO}}$, correction is made for backpressure of carbon monoxide due to significant concentrations of blood carboxyhemoglobin. Helium is measured with a thermal conductivity meter, and carbon monoxide with an infrared detector or an electrochemical analyzer. Reproducibility of this test may be poor under some conditions and it is affected by changes in ventila-

tion diffusing capacity ratios, but the test offers the potential of detecting changes in the blood-air interface (such as alveolar edema) that might otherwise go undetected.

Oxygen consumption can be measured at rest and during exercise on a constant-load bicycle ergometer or a treadmill at a level producing a specified percentage of predicted maximal oxygen consumption.[4] Expired air is collected and measured with a spirometer to determine total expired volume, and samples are analyzed for oxygen and carbon dioxide.

Carboxyhemoglobin concentration [COHb] can be estimated with the method of Jones and co-workers.[35] The subject holds a deep breath for 20 s to allow equilibration of carbon monoxide between alveolar air and blood and then expires a sample of that air into a container. The air carbon monoxide concentration may be directly related to [COHb]. The test can be performed before exposure in an environmental chamber to help to verify that the subject has not received inordinate ambient pollutant exposure.

Biochemical studies on human blood samples have been used to measure response by Buckley et al.,[15] who reported changes in red cells and serum of men after a single acute exposure to ozone at 0.50 ppm for 2.75 h. Red-cell membrane fragility and glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities were increased, and red-cell acetylcholinesterase activity and reduced gluathione were decreased. Red-cell glutathione reductase (GSSRase) activity was not significantly altered; however, serum GSSRase activity was significantly decreased, and serum vitamin E content and lipid peroxidation were significantly increased.

A number of investigators[11,46,48] have argued that behavioral changes are more prevalent than frank physiologic or clinical changes as

effects of air pollutants. If the ability to perform routine tasks, such as operating an automobile or a complex piece of machinery, is compromised by pollutant exposure, the setting of air pollution standards should take such effects into consideration.

In one study,[28] human subjects were tested in a controlled-environment chamber with a high (summer) temperature and with ozone, nitrogen dioxide, and carbon monoxide as pollutants. Performance on a divided-attention task given at the end of the exposure period and the subjects' heart-rate variability (as a potential psychophysiologic measure of attention) were evaluated. The subjects displayed a significant decrement in peripheral attention associated with increased ambient temperature. Effects attributable to pollutant gases were variable.

Subjects exposed to ambient oxidant pollution or to controlled oxidant pollution in a test chamber express their response by symptoms, as well as by physiologic changes. Because of their subjective nature, little attention has been given to symptoms in overall assessments of effects; however, with systematic collection, these experimental data lend themselves to semiquantitation.

In the study by Hackney et al,[28a] the investigators proposed a method for consistent gathering of symptom data by means of a checklist, wherein symptoms are scored (according to predetermined criteria of severity) for three different periods during and after exposure. The sum of the scores for all symptoms from all periods gives a total symptom score, or discomfort index, for the 24-h period after the start of exposure. In their study, symptom scores for 42 subjects exposed to ozone at 0.25, 0.37, and

0.5 ppm revealed a dose-response relationship for higher doses that approximated the relationship demonstrated by objective pulmonary function. These results suggest that semiquantitation of symptoms is feasible in controlled pollution-exposure studies and may be a useful clinical investigative tool.

Experimental Design Considerations

Multiple stresses may be important in assessing functional and adaptive capabilities or people. An exposure protocol can be designed to simulate as closely as possible the ambient exposure of a group likely to have significant exposure, such as people working outdoors on a smoggy summer day. A 2-h exposure period is realistic, in that high ambient pollutant concentrations usually persist about that long. Other exposure periods should be chosen to simulate different pollutant episodes. Specified amounts of exercise can serve as a tool in experimental design to provide additional stress. Intermittent light exercise (sufficient to approximate a doubling of minute volume) during exposure produces a realistic degree of ventilation (to which pollutant dose is approximately proportional) during typical work. Increased temperature is another stress factor that is often present during oxidant air pollution episodes and so can be introduced into the experimental situation. The design can provide for successive days' exposures, in that deleterious effects of exposure may be cumulative. These requirements can be incorporated into a protocol in a cost-effective manner that tests several subjects on a given day. However, with a single chamber of limited size, this requires staggered exposure and testing periods, precluding "blind" studies or control measurements on the same day. Thus, sham control runs (identical protocols with exposure to purified air) precede the pollutant exposure, so that reliable baseline values of the measured characteristics can be obtained.

"Blind" studies with chemical substances that have a characteristic odor or irritant effect are difficult to manage. If, as with ozone, the odor sense tends to diminish, then "odor sham" protocols are possible. However, keeping the subjects and the investigators (other than the safety officer) truly uninformed about the nature of a particular experiment is not a trivial problem.

Cyclic variations in measurements are potentially important.[37] Adequate information is not available, for example, on normal variation over time in tests of human physiologic function. This presents a problem in designing not only short-term experiments, but especially longer-term studies. Detailed data on stability of a given measurement over periods of minutes, hours, days, weeks, months, and even years would improve rational experimental designs. Variations in measurements with changing seasons, especially summer versus winter, might be expected, because of the effect of large temperature differences on physiology and life style. Interactions may be important in design and data interpretation. The likelihood of recent prior exposure to high oxidant concentration is clearly a function of the summer season and geographic location.

There is a definite need for dose-response information on specified groups of people, if frequency distributions of responses are to be estimated. Obtaining and using such information present numerous problems, such as selection of appropriate groups and their size; the need to include persons with disease, as well as the very young and very old; and extrapolation of the data to the total population. With separate dose-response information on representative samples of major population groups, a frequency distribution of responses at each pollutant concentration might be determined by weighting

the mean change for each group according to the general populations they represent. Information on frequency distribution of responses is necessary for any cost-benefit analysis that will require quantitative estimates of effects in terms of dollars.

With due regard to the factors just discussed, a test series may reasonably be designed to support or reject the hypothesis that no effects of realistic pollutant exposure will be detected in volunteer subjects. Results supporting the hypothesis would be useful to regulatory agencies in setting air pollution standards. If minimal effects are found in a group of "normal" subjects, an effective experimental strategy is to test a group of specified "hyperreactive" subjects, characterized by a prestudy history of cough, chest discomfort, or wheezing associated with allergy or exposure to air pollution.

Experimental data can be subjected to repeated one-way variance analyses. Post hoc comparisons with the Newman-Kuels test[58] are made when significant differences among test conditions are found. A few significant differences due to random variation may be found, because of the number of statistical comparisons usually being made; therefore, all observed, statistically significant changes must be examined critically for physiologic significance.

Ambient-Air Studies

Experiments can be conducted with well-specified ambient air, if a suitable chamber is in a highly polluted area and if ambient air can be introduced into the chamber without significant pollutant losses. Other runs with clean air and matched temperature and humidity serve as controls. Alternatively, mobile health-testing laboratories with air monitoring capabilities can go to polluted areas of interest.

Detection of short-term cardiopulmonary effects of ambient oxidant pollutant exposure is complicated by several factors. The physiologic measures of such effects exhibit significant inherent variability, which may mask the relatively small changes expected. The atmosphere contains many potentially hazardous materials, both natural and man-made, in continuously varying concentrations. Not all these substances have been identified, and many that have been identified cannot be adequately monitored. Other environmental factors, such as temperature and humidity, may influence physiologic function independently and thus complicate the detection and interpretation of pollutant effects. Variations in concentration of any given pollutant are usually closely linked to variations in concentrations of other pollutants, and this makes it difficult to assess the toxicity of a single component of the ambient mixture. These problems have led to the assertion that ambient-exposure study results cannot be interpreted reliably, owing to the incompleteness of both the atmospheric and the biologic information and the inability to control or allow for all interfering variables. This assertion is correct in principle, but neglects the possibility of obtaining valuable partial answers to health-effects questions through ambient studies. Such improved understanding of the effects of ambient oxidants should be attainable through improved atmospheric monitoring; improved physiologic, biochemical, and clinical evaluation of exposed subjects; application of findings from concurrent controlled-exposure studies; and combination of ambient- and controlled-exposure studies.

REVIEW AND DISCUSSION OF PUBLISHED DATA

The data base on health effects of photochemical oxidants and ozone was reviewed by the Subcommittee on Ozone and Other Photochemical Oxidants

in a report prepared in September 1974 for the Committee on Public Works, U.S. Senate.[2] The following discussion repeats some of the material in that report, to exemplify the need for further work, including controlled human studies.

The federal primary ambient air quality standard for photochemical oxidants is 160 $\mu g/m^3$ (0.08 ppm), a maximal 1-h average concentration not to be exceeded more than once per year.[56] Air Quality Criteria for Photochemical Oxidants[1] catalogs and describes the overall data base for present standards. In information received by the Subcommittee (February 23-24, 1974), the following evidence was cited as the basis on which the EPA set the standards:

- When high-shcool cross-country runners were exposed for 1 h to photochemical oxidants at 0.03 - 0.3 ppm, their performance decreased with increasing concentration.[57] A statistical test for threshold values (regression using "hockey stick" functions) applied to these data gives a threshold estimate of 0.12 ppm, with a 95% confidence interval of 0.067 - 0.163 ppm.[1,5,30,44,57]
- At short-term maximal peak oxidant concentration of 490 $\mu g/m^3$ (0.25 ppm), subjects with asthma begin to experience significantly more attacks. These maximal daily peaks may occur with a maximal hourly average concentration as low as 300 $\mu g/m^3$ (0.15 ppm). Eight people sensitive to smog experienced increased attacks at oxidant concentrations corresponding to those at which plant damage occurs (8-h average, 200 $\mu g/m^3$, or 0.10 ppm).[1,49]
- At short-term peak oxidant concentration of 196 $\mu g/m^3$ (0.10 ppm) and above, humans begin to experience eye irritation. These

peaks would be expected to occur with maximal hourly average concentrations of 50-100 $\mu g/m^3$ (0.025-0.05 ppm).[45,47]

- At short-term ozone concentration of 160 $\mu g/m^3$ (0.08 ppm) for 3 h, experimental animals (mice) exhibited increased susceptibility to laboratory-induced bacterial infections. A firm dose-response relationship was established for this effect.[18,19]
- Brinkman et al.[14] found that, at short-term ozone concentration of 392 $\mu g/m^3$ (0.20 ppm) for 1-2 h, experimental animals had increased sphering of red blood cells. Similarly, humans had increased sphering after 30 min of exposure to 490 $\mu g/m^3$ (0.25 ppm).
- Brinkman et al.[14] also found that, at long-term ozone concentration of 392 $\mu g/m^3$ (0.20 ppm) for 5 h/day for 3 weeks, structural changes in the nuclei of heart muscle fibers were produced in adult rabbits and mice. The fibers reverted to normal a month after exposure.

The EPA used the data from the first three studies listed to obtain the value of 200 $\mu g/m^3$ (0.1 ppm) for a maximal 1-h concentration--the lowest concentration at which measurable human effects are generally observed. No formal methodology was used in making this selection; it was derived as a best judgment by EPA officials.

Similarly, no published record exists to describe the procedure used in selecting 0.08 ppm as the national primary standard for photochemical oxidants. It is the recollection of EPA officials that the standard was set midway between the highest average background concentration of ozone (0.06 ppm) and 0.1 ppm, giving a 20% "margin of safety."

There are obvious inadequacies in the established values. First, it was assumed that the oxidant standard is a surrogate standard for photochemical oxidants, with ozone as the indicator. However, it is clear that very low concentrations of specific irritants, such as peroxyacetylnitrate (PAN), are sufficient to cause eye irritation. Second, some of the statistical techniques used to determine the lowest concentration at which effects are observed were inconsistent and undocumented. A "hockey stick" function[5,44] was sometimes used to find an effect threshold.

The inadequacy of the technical data base on photochemical oxidants was realized from the start by EPA officials; Barth et al.[5], describing the situation in 1971, stated that "many bits of information required to place the present National Ambient Air Quality Standards on an irrefutable and unassailable scientific basis are not yet available."

Exposure to Ozone

Studies before 1970 are reviewed in Air Quality Criteria for Photochemical Oxidants.[1] Details of selected controlled human studies repeated later are given below.

Bates and Hazucha[10a] measured significant changes in lung function (decrease in maximal flow rate at 50% of the vital capacity, maximal transpulmonary pressure, and increase in total pulmonary resistance) in 10 normal male subjects aged 23-35 years (including two smokers) exposed to pure ozone at 0.75 ppm for 2 h. Two of the three subjects who exercised intermittently at twice the resting volume showed accentuated effects. In a separate study by Hazucha et al.[32] on effects of short-term exposure, significant changes in lung function (decreases in forced vital capacity, forced expired volume at 1 s,

maximal flow rate at 50% of vital capacity, and maximal midexpiratory flow rate; and increases in closing capacity and residual volume) were found in 12 normal males aged 23.6 $\pm$ 0.7 years (including six smokers) exposed to pure ozone at 0.75 and 0.37 ppm for 2 h during alternating rest and exercise periods. The higher concentration affected smokers more than nonsmokers, whereas the lower concentration affected smokers and nonsmokers similarly. In these two studies, most subjects complained of cough, chest tightness, and substernal soreness. A few also had pharyngitis, dyspnea, and wheezing.

Hazucha[31] also measured small decreases in the same lung functions as above in three normal male subjects of mean age 20 years (nonsmokers) exposed to pure ozone at 0.25 and 0.56 ppm for 2 h during alternating 15-min periods of rest and exercise. Although these decreases were not statistically significant, in view of the small number of subjects involved, they were consistent and in general agreed in value with dose-response curves drawn from data at 0.75 and 0.37 ppm (Figure 9-1). The subjects coughed and complained of chest tightness, substernal soreness, increased salivation, and expectoration of mucus. The studies just described[9,31,32] were conducted in the Montreal chamber facility with general conditions of: temperature, 21-23 C; humidity, 42-49% inside background air; and two or more Mast coulombic meters for oxidant monitoring.

Studies by Hackney et al.[28a, 28b, 28c] used a protocol designed to simulate summer exposure in the Los Angeles southern coastal air basin and included the additional stresses of heat, intermittent exercise, and repeated exposures. Careful attention was given to environmental control, pollutant monitoring and generation, and subject selection. Four male

subjects aged 36-49 years (including one cigarette smoker) judged by subjective criteria to have normally reactive airways completed this protocol. No obvious effects -- as assessed by clinical response and measures of respiratory, cardiac, and metabolic functional change -- were noted after exposure for 4-5 h to 0.5-ppm ozone, 0.5-ppm ozone and 0.3-ppm nitrogen dioxide, or 0.5-ppm ozone, 0.3-ppm nitrogen dioxide, and 30-ppm carbon monoxide. A second group of four, aged 29-41 years (including two cigarette-smokers), who had previously experienced clinical bronchospasm and were judged by subjective criteria to have hyperreactive airways (history of developing symptoms during light activity in smog or history of asthma) developed clinical discomfort and were unable to complete the protocol. Exposed to ozone at 0.5 ppm for 4-5 h, this group developed marked changes in pulmonary mechanics and gas distribution. Some effects were later found after exposure to ozone at 0.37 ppm, but not at 0.25 ppm. In light of the marked clinical effects, the protocol was modified, and a third group of seven subjects, aged 22-36 years (including two smokers), judged to have normally reactive airways, were studied. This group, exposed to ozone at 0.5 ppm for 2 h, showed only minimal effects on the first day of exposure; however, five of the seven showed significant effects on a second exposure day. A fourth group of seven subjects, aged 22-41 years (including three cigarette-smokers), exposed for 2 h to 0.25-ppm ozone, 0.25-ppm ozone and 0.3-ppm nitrogen dioxide, or 0.25-ppm ozone, 0.3-ppm nitrogen dioxide, and 30-ppm carbon monoxide showed no obvious effects. Of seven subjects, three were judged on the basis of subjective criteria to have hyperreactive airways and four were deemed to have normally reactive airways. A fifth group of five subjects, aged 27-41 years (including one cigarette-smoker), was exposured to 0.37-ppm ozone for 2 h. Important changes with exposure were found in most physiologic measures. However, one subject showed substantial changes in measures of

lung mechanical function, which became worse on the second day of exposure. Findings from this series of studies indicate that there is a wide range of sensitivity to photochemical pollutants and that more sensitive people develop significant symptoms, biochemical changes, and respiratory function decrease under exposure conditions similar to those experienced during pollution episodes. The studies just described[28a,28b,28c] were conducted in the Rancho Los Amigos Hospital chamber facility with general conditions of: temperature, 31C; humidity, 35%; purified background air; and chemiluminescence and neutral potassium iodide montoring for oxidants.

Comparison of health effects of exposure to ozone[7,28b,28c,31] suggests that Canadians are more reactive than southern Californians. These results are summarized in Figure 9-1. Experimental methods and subject responses were compared further in a cooperative investigation of this apparent reactivity difference. In studies conducted in California, four Canadians and four Californians were exposed to ozone at 0.37 ppm in purified air at 21 C and 50% relative humidity for 2 h with intermittent light exercise.[27a] Exposures to purified air alone served as controls. Methodologic differences sufficient to explain different results of previous studies were not found. Subject responses were similar to those observed previously--Canadians on the average showed greater clinical and physiologic reactivity to exposure than did Californians, who were no more than minimally reactive. Canadians also showed larger increases in red-cell fragility after exposure. These results support the existence of a real difference in reactivity between the Californians and Canadians studied to date. Although the number of subjects tested in the cooperative study is small, the good agreement between these results and previous results in the separate laboratories allows considerably increased confidence in direct comparison of the previous results

from Los Angeles and Canada, providing a considerably larger data base from which to judge relative reactivity.

If the difference in response to ozone between Canadians and southern Californians studied is accepted as real, a hypothesis of adaptation in southern Californians is supported. Further support of the hypothesis requires demonstration that the subjects tested are truly representative of larger population groups residing in the same areas and that identifiable factors other than adaptation are not sufficient to explain the observations.

In studies by Kerr *et al.*,[38] 20 healthy adults -- nine males and one female, aged 21-60 years (including 10 smokers and 10 nonsmokers) -- were exposed to ozone at 0.5 ppm for 6 h in an environmental chamber. During this period, they engaged in two 15-min medium exercise sessions (100 W at 60 rpm) on a bicycle ergometer. Symptoms of dry cough and chest discomfort, after ozone exposure, were more commonly noted in nonsmokers. They also had a significant decrease in dynamic compliance after exposure. Subjects who experienced symptoms, in general, were the ones who developed objective evidence of decreased pulmonary function. Chest discomfort ranged from tightness on full inspiration to generalized chest pain accentuated by exercise, cough, and irritation of the nose and throat. Significant changes from control values for the group as a whole after ozone exposure were observed for several pulmonary function tests: specific airway conductance (SGaw), pulmonary resistance (R_L), FVC, and FEV_3. No significant change was observed with respect to diffusing capacity ($D_{L_{CO}}$), static lung compliance (C_{st}), or the various tests derived from the nitrogen elimination rate. When the smokers were considered as a separate group, no significant decrease in pulmonary function was observed, although some

individual smokers had decreases in pulmonary function. These studies[38] were conducted in the Baltimore chamber facility with general conditions of: temperature, 24 C; humidity, 45%; purified background air; and two Mast coulombic meters for oxidant monitoring.

Folinsbee et al.[64] tested the response of 28 subjects after ozone exposure to three stages of ergometer exercise with loads adjusted to 45, 60, and 75% of maximal aerobic power. The subjects were exposed to ozone at 0.37, 0.40, or 0.75 ppm for 2 h, at rest or while exercising intermittently -- 15 min of rest alternated with 15 min of exercise at a workload sufficient to increase ventilation by a factor of 2.5. These studies were conducted in a plexiglass chamber (Toronto). Oxidant monitoring was with a coulombmetric analyzer (Mast), which was checked periodically against neutral buffered potassium iodide. Neither submaximal exercise oxygen consumption nor minute ventilation was significantly altered after ozone exposure at any concentration. The primary response was an alteration of exercise ventilatory pattern. An increase in breathing rate ($r=0.98$) and a decrease in tidal volume ($r=0.91$) were correlated with the dose of ozone, calculated as the volume of ozone inspired during exposure. It was concluded that through its irritant properties, ozone modified normal ventilatory response to exercise and that this effect was dose-dependent.

Rummo et al.[48a] exposed 22 male volunteers, aged 19-27, for up to 4 h to ozone at 0.4 ppm in relatively clean ambient air or to ambient air alone. Subjects were seated during exposure, except for two 15-min exercise periods on a bicycle ergometer at 700 kg-m-min exercise that about doubled the heart rate and quadrupled the ventilation volume. After 2 h of ozone exposure, there was a significant change ($p < 0.05$) in FVC, MMF, and airway resistance (Raw). Several other measures (FEV_1, $\dot{V}_{50}$, and $\dot{V}_{25}$) were lower after 2 h of exposure, but the statistical significance was borderline.

However, after 4 h of exposure, all flow measures were significantly decreased, compared with controls. After 4 h, Raw increased, FVC decreased further, and FEV_1 decreased significantly. Residual volume, functional residual capacity, and total lung volume did not change as a result of the ozone exposure.

Kagawa and Toyama[36] have reported on the results of limited studies involving four normal male subjects exercising while exposed to ozone at 0.9 ppm for 5 min. A significant decrease in SGaw was found during exposure and after 5 min of recovery.

In all reported studies,[9,28a,b,c,31,32,38,48a,64] an association between symptoms and changes in lung function was usually found. In general, people who noticed cough and substantial tightness first were the ones who developed the greatest defect in function. Ozone-induced defects in function were not usually found in the absence of definite symptoms of ozone-induced respiratory irritation.

It would be desirable at this point to discuss mechanistic interpretation of the lung function measures -- such as FEB_1, delta nitrogen, and airway resistance -- that have been reported to change with ozone challenge. Unfortunately, although such measurements as FEV_1 give an easily obtained, relatively reproducible evaluation of overall lung mechanical performance, they provide little information on the mechanisms responsible for any observed change. Also, the measurement of the slope of the alveolar nitrogen plateau (delta nitrogen) cannot be interpreted beyond saying that it reflects the uniformity of ventilation distribution. Airway resistance and specific airway conductance are thought to be sensitive measures of constriction of large airways. Results of animal experiments provide additional information about mechanisms of action of oxidant pollutants on the respiratory system. These are discussed in Chapter 8.

In interpreting the results of human experimental studies with pure ozone in relation to the oxidant standard, it must be remembered that the other oxidants ordinarily present in smog were absent. Conceivably, a larger difference may be necessary between the lowest concentration of pure ozone at which an observed effect occurred and the air quality standard than between the lowest concentration of oxidant mixtures and the standard.

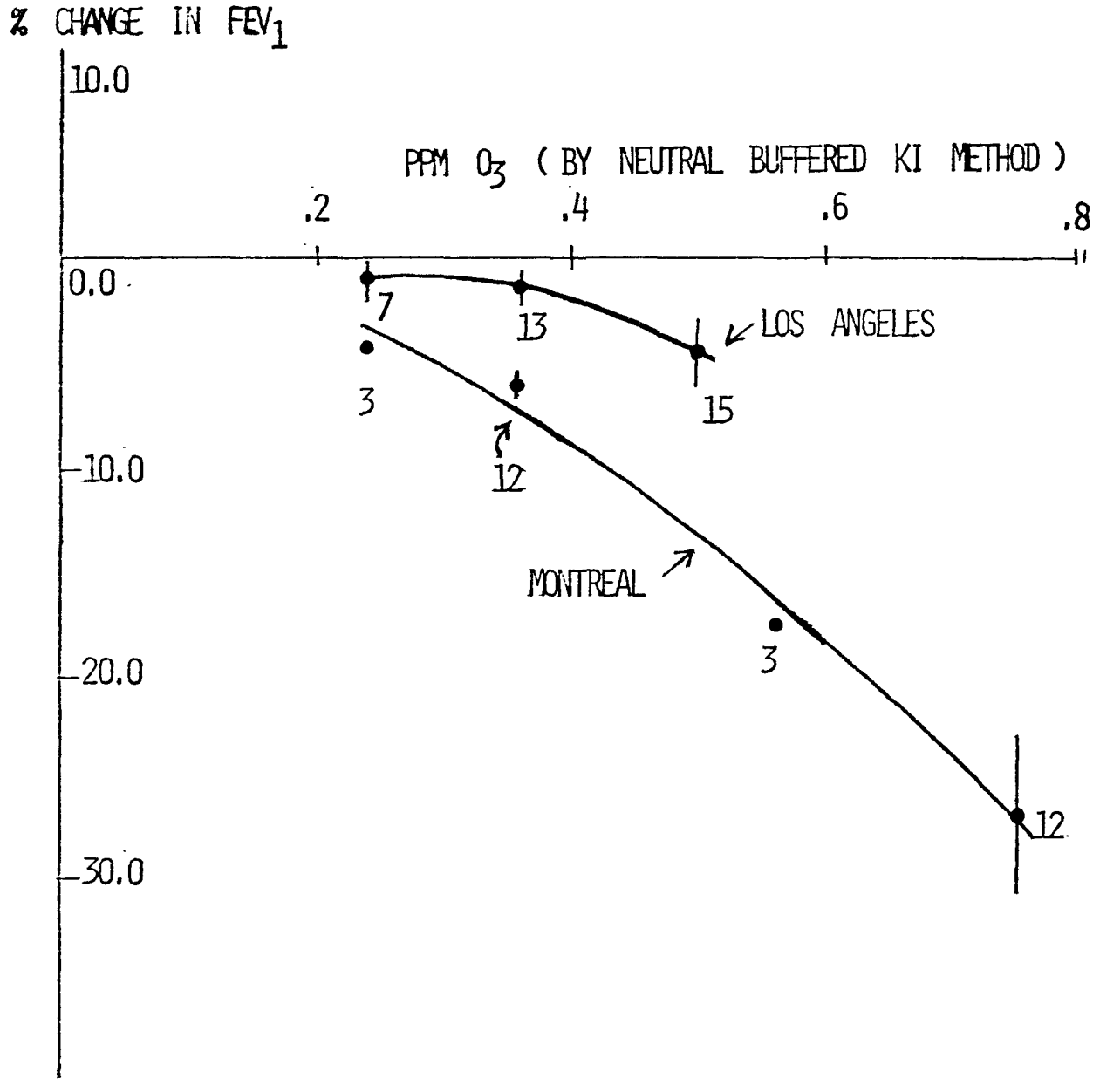
The newer experimental studies described above[10,28a,28b,28c,31,32,36,38,48a,64] show that significant adverse health effects occur in humans at ozone concentrations of 0.37 ppm and higher. Some limited studies[31] show evidence of human health effects of exposure to pure ozone at concentrations as low as 0.25 ppm (see Figure 9-1). The uncertainties of extrapolating ozone effects to oxidant effects, of dose estimation, of measurement, and of subject sensitivity must be kept in mind when comparing the new data with the standard.

Exposure to Ozone and Other Pollutants

Hazucha[31] and Bates and Hazucha[10] reported an enhanced effect between ozone and sulfur dioxide in controlled-exposure studies of airway responses in humans. Hazucha studied subjects only in the resting state; Bates and Hazucha, during rest and exercise. With various tests of ventilatory function--including FEV_1, midmaximal flow rate, and maximal expiratory flow rate at 50% of vital capacity -- the investigators showed that healthy male college students experienced no effect of sulfur dioxide at 0.37 ppm, a 10% decline in function with ozone at 0.37 ppm, and a 20-40% decline in function with a combination of sulfur dioxide at 0.37 ppm and ozone at 0.37 ppm. The effect of ozone alone on human function was not clearly mainifested until subjects had been exposed for 2 h; the effect of the combination of ozone and sulfur dioxide was apparent within 0.5 h. The maximal effect was observed in the first 30 min

Figure 9-1. Behavior of FEV_1 in response to ozone expressed as percentage change from the appropriate control (SHAM) experiment. Smokers and nonsmokers combined. Exposures 2 h, with intermittent light exercise, except some Los Angeles exposures at 0.5 ppm for 4 h with intermittent exercise. Points of error bars represent mean response (ΔFEV_1 postexposure vs. control) ± 1 standard error. Curves represent best second-order polynominal fit to mean points. Numbers adjacent to points represent sample sizes: L.A., 7 at 0.25 ppm, 13 at 0.37 ppm, 15 at 0.5 ppm; Montreal, 3 at 0.25 ppm and 0.56 ppm (error bars not given, because of small sample size), 12 at 0.37 ppm and 0.75 ppm. Mean values for L.A. and Montreal 0.37 ppm x 2 h exposures are significantly different. $t = 4.15$. $p < 0.01$. Equations for dose-response curves: L.A., second-order fit to 3 data points, $(\Delta FEV_1\%) = -7.524 + 46.546\ (\text{ppm } O_3) - 81.795\ (\text{ppm } O_3)^2$; Montreal, second-order fit to 4 data points, $(\Delta FEV_1\%) = 2.147 - 13.229\ (\text{ppm } O_3) - 35.383\ (\text{ppm } O_3)^2$. NOTE: These equations are only for the concentration ranges of the data.

FIGURE 9-1


% CHANGE IN FEV_1
10.0
PPM O_3 (BY NEUTRAL BUFFERED KI METHOD)
.2
.4
.6
.8
0.0
7
13
LOS ANGELES
3
15
12
-10.0
MONTREAL
3
-20.0
12
-30.0

after cessation of exposure. Care was taken to use scrubbers on the instruments that were used to measure each gas concentration. The effect of the combined gases was greater than the sum of the effects of the two gases administered separately.

Adult male volunteers were exposed to purified air,[28a,28b,28c] to ozone alone, or to ozone in combination with nitrogen dioxide and carbon monoxide. No additional effects were detected when nitrogen dioxide at 0.3 ppm was added to ozone. The addition of carbon monoxide at 30 ppm to the ozone-nitrogen dioxide mixture produced no additional effects, other than a slight increase in blood carboxyhemoglobin content and small decreases in psychomotor performance, which were not consistent in different subject groups.

Exposure to Peroxyacetylnitrate

In 1965, Smith[52] reported increased oxygen uptake during exercise in college students exposed to PAN at 0.3 ppm. The average increase was 2.3%. More recently, Drinkwater *et al.*[21] and Ravin *et al.*[62] found no significant physiologic change attributed to PAN exposure. They tested 20 young men (smokers and nonsmokers) for maximal aerobic power (treadmill walk) in a 35 C environment under four ambient air conditions: filtered air, carbon monoxide at 50 ppm, PAN at 0.27 ppm, and carbon monoxide and PAN. No significant physiologic effects were noted during the PAN exposure for smokers or nonsmokers. Maximal aerobic power was not affected by any pollutant condition. Heat stress was more effective than any pollutant condition in reducing work capacity, and a 4.3% decrease in maximal aerobic power in a 35 C environment, compared with a 25 C environment, was greater than the differences in aerobic power found between filtered air and any of the pollutant conditions.

Other Experimental Studies

Holland et al.[34] studied 14 subjects under conditions of short-term exposures to irradiated automobile exhaust. The environmental conditions simulated the "moderate" smog episodes in the Los Angeles air basin. Oxidant concentrations were reported as 0.22-0.27 ppm (on the basis of the alkaline potassum iodide method). No significant changes attributed to exposure were found in reaction time, vital capacity, work performance, or oxygen consumption.

Henschler[60] reported that the characteristic pungent odor of ozone was detected instantaneously at low concentrations (less than 0.02 ppm), depending on individual sensory perception acuity, and perceived at higher concentrations (0.05 ppm) for an average of 5 min. At higher concentrations, the odor was perceived as stronger and persisted for an average of 13 min.

Eye irritation has been a common complaint of people exposed to photochemical air pollution.[33] Attempts to investigate this experimentally have encountered problems, because of the subjective nature of the human response and the multiphasic photochemical reactions involved. Human studies conducted until 1970 on eye irritation are cataloged and discussed in National Air Pollution Control Administration Publication AP-64, Air Quality Criteria for Hydrocarbons.

Several studies indicate that the major photochemical products that cause eye irritation are acrolein, PAN, and peroxybenzoylnitrate (PBzN).[50,54,61] Ozone, the principal contributor to ambient oxidant concentrations, is not an eye irritant. This is an important observation, because it might have been assumed that lacrimation would correlate with respiratory effects. In fact, however, ozone concentrations can be high enough to cause considerable respiratory effects without any irritation of the eyes having been noted. Heuss and Glasson[61] state that the potency of PBzN as an eye irritant is 200 times

that of formaldehyde. In studies by Heuss et al,[33] PBzN was formed in parts-per-million amounts by irradiating automobile exhaust in laboratory apparatus. When some aromatics were added to a low-aromatic gasoline, PBzN and greatly increased eye irritation resulted.

Wilson et al.[63] measured irradiated automobile exhaust and pure organic compounds and found that the addition of sulfur dioxide, although it increased aerosol formation, decreased eye irritation in many of the hydrocarbon - NOx systems studied.

A number of components of ambient oxidant mixtures are discussed in Chapter 3. Further detailed characterization studies of ambient particulate pollution may suggest that some of these compounds are present in the organic fraction in quantities likely to be detrimental to human health. Further controlled health-effects studies might be accomplished with irradiated and diluted automobile exhaust or exposure to pure specific compounds.

Limitations of Controlled Human Studies

It is apparent that controlled human experimental studies are needed, but are cumbersome and costly. Other limitations include restrictions as to the number of measurable responses and the fact that individual pollutants are usually studied, rather than ambient mixtures. In addition, healthy subjects are usually studied, rather than sensitive population groups. The results of these studies are applicable to acute effects; but their relation, if any, to chronic effects is not known.

Ethical considerations impose design requirements that increase the cost and complexity of controlled human studies. For example, prudent operational guidelines for such studies could include:

- The investigators serving as the first subjects for each series of exposure studies.
- Pollutant exposure concentrations selected so as not to exceed documented ambient concentrations.
- The exposure environment constantly monitored by the technician who is operating the pollutant generating equipment.
- The presence of a physician.
- Monitoring of subjects electrocardiographically from outside the chamber.
- Direct observation of subjects at all times by viewport or closed-circuit television.
- Frequent checks by the attending physician and the chamber engineer for hazardous conditions and, if they encounter any, application of corrective measures or halting of the study.
- Physician screening of prospective volunteers.
- Obtaining of informed consent after the subject is given a full explanation of the experimental procedures, including known risks and discomforts. The form would describe the procedures and risks fully and specify that the subject may withdraw from the study at any time.

SUMMARY

Convincing new information on the health effects of oxidant exposure has emerged from controlled studies on humans, from which tentative dose-response curves have been constructed. The new data show statistically significant reduced pulmonary function in healthy smokers and nonsmokers at ozone concentrations at and above 0.37 ppm for 2-h exposures. Other gases

and aerosols found in an urban atmosphere were not present in these experiments. Some studies suggest that mixtures of sulfur dioxide and ozone at a concentration of 0.37 ppm are more active physiologically than would be expected from the behavior of the gases acting separately.

Wide variation in response among different individuals is a general finding in studies of oxidants, as well as other pollutants.

Undesirable health effects of oxidant air pollution exposure are increased by exercise, and many people apparently limit strenuous exercise voluntarily when oxidant pollution is high.

Safety, ethical, and legal considerations require that the utmost care be exercised in human experimentation. The risk inherent in this work can be minimized by taking reasonable precautions while ensuring the satisfactory performance of the study.

RECOMMENDATIONS

Further studies are needed to give better dose-response information and to provide a frequency distribution of the population response to oxidants alone and in combination with other pollutants at various concentrations. Such studies should include the effects of mixed pollutants over ranges corresponding to the ambient atmosphere. With combinations of ozone and sulfur dioxide, the mixture should be carefully characterized to be sure of the effects of trace pollutants on sulfate aerosol formation. The design of such studies should consider the need to use the information for cost-benefit analysis and for extrapolation from animals to humans and from small groups of humans to populations. Recent research has indicated the possibility of human adaptation to chronic exposure to oxidants. Further study is desirable.

Studies are needed to clarify the importance of age, sex, ethnicity, familial elements, nutritional factors, and pharmacologic agents in determining response to oxidants. Because people with lung disease are thought to be more susceptible to oxidant pollutants, exposure studies are needed to quantify this. Better methods for measuring or estimating the actual dose of oxidants absorbed by each subject are needed. The usual time variation in measures of human response should be evaluated per se, because this information is needed to optimize experimental design.

More information is needed before rational guidance can be given about limiting exercise during periods of high oxidant pollution.

Standards for the exposures of humans to controlled atmospheres should be discussed by national groups and agencies, such as the National Academy of Sciences, the American Medical Association, and the National Institutes of Health.

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Chapter 10

EPIDEMIOLOGIC STUDIES

A variety of epidemiologic studies attempting to associate various characteristics of human health and functioning with daily concentrations of photochemical oxidants have been carried out during the last two decades, primarily in the Los Angeles air basin. A few of these have shown clear-cut associations involving large segments of the population. Other studies related to both the same and different health indicators have failed to demonstrate any consistent association. Failure to establish clear-cut associations may well be inherent in some aspects of the epidemiologic method. Epidemiologic studies have the advantage of being focused on the real world, using human populations in their normal setting. This permits identification of both long-term and short-term variations in health and function that can be correlated with pollutant exposures. It also permits identification of susceptible groups within the general population that may be at greater risk. Epidemiologic studies, however, have two main disadvantages: the health indexes used must inevitably be relatively crude, as opposed to controlled laboratory conditions; and it is extremely difficult in the real world to isolate the effect of one environmental factor in the presence of numerous other independent and dependent variables, which may be synergistic with or antagonistic to the environmental factor under study--e.g., other air pollutants, meteorologic factors, socioeconomic status, occupational exposures, personal health habits, and cigarette-smoking. Within these limits, some definite conclusions can be drawn as to the association (or lack of it) of some health indicators with photochemical oxidant pollution. Because of the lack of epidemiologic information in some critical areas, use

must be made of clinical and toxicologic studies on photochemical oxidants, both in setting ambient air standards and in planning future epidemiologic studies.

MORTALITY

Death is obviously the most clear-cut and significant end point for determining the effect of an environmental challenge on health. It rarely has a single, isolated cause, however, even in apparently simple events like automobile accidents, homicide, and suicide. Careful investigation will usually reveal a constellation of factors that contributed to the final event. Documented episodes of deliberate or inadvertent exposure of a sizable population to a high concentration of a known toxic substance are extremely rare, and all attempts to link death with ambient air pollution at normal concentrations must consider a multitude of factors. Furthermore, these factors often vary from person to person and from situation to situation. Among the factors that must be considered are the age and state of health of the person affected, the presence of coexisting disease states (likely for many people), the activity being pursued by the person at the time of and just before death, and individual biologic variation in response to different types of stress. Other factors that must be considered in relation to the specific challenge are the type of pollutant, the presence of other pollutants (always a factor in epidemiologic studies of populations in natural environments), the duration of exposure at various concentrations, the frequency and degree of previous exposures to similar pollutants that may initiate either tolerance or hypersensitivity to the offending agent, and the meteorologic conditions under which exposure may take place. The last factor is often shown to play an overwhelming role in determining the outcome of the challenge.

Several mortality studies on different population groups have been conducted, principally in the Los Angeles area. Studies by the California Department of Public Health (reported in 1955, 1956, and 1957)[1] showed a marked increase in daily mortality among Los Angeles County residents aged 65 and older during a period of high photochemical oxidant concentrations in a 2-week period in September 1955. An examination of temperatures during this period showed that the increase in deaths occurred immediately after a marked increase in temperature; mortality returned to normal before oxidant concentrations had returned to those prevailing before the episode. Although the photochemical oxidant concentrations were high immediately before, during, and after the heat wave, the daily mortality decreased when temperatures dropped. During other periods when daily mortality in citizens over 65 was compared with both temperature and oxidant concentrations, no consistent pattern could be discerned, nor could a pattern be seen at other times when oxidants rose to medium and high concentrations without an accompanying increase in temperature. It must be concluded that heat rather than photochemical oxidant exposure, precipitated the increase in fatalities in the population studied. Nevertheless, the possibility cannot be dismissed that the concomitant high oxidant concentrations increased the death toll. Such synergistic actions between air pollutants and weather are well known.[2]

Heat has long been known to be one of the greatest stresses to which an elderly person can be subjected, and heat waves in New York, as well as Los Angeles, have been regularly shown to be followed by a tripling or quadrupling of daily deaths among persons over 65, especially before air conditioning became prevalent. Unfortunately, high temperatures are often associated with high oxidant concentrations in the Los Angeles basin.

In an attempt to isolate the effects of these two environmenta characteristics Oechsli and Buechley[3] studied the mortality associated with three Los Angeles heat waves, in 1939, 1955, and 1963. It was assumed that less photochemical oxidant pollution was present in 1939 (although no measurements were made at that time), and yet there was an equally high mortality among elderly persons in 1939 and 1955. Temperatures were similar in each of the three heat waves. A lessened mortality was observed during the 1963 heat wave, perhaps because of the increasing use of air conditioning between 1955 and 1963. The authors concluded that the high photochemical oxidant concentrations did not augment the mortality effect of high temperatures.

Residents of nursing homes were a special group included in the California Department of Public Health study.[1] They were considered of special interest because of the assumed presence of chronic illness in most of them and their possible greater susceptibility to photochemical oxidant pollution. All nursing homes in Los Angeles County containing 25 or more beds were included, and daily mortality, transfer to a hospital because of worsening disease, maximal daily temperatures, and the occurrence of smog-alert days with ozone concentrations of 590 $\mu g/m^3$ (0.3 ppm) or higher were considered. Again, the striking effect on mortality during heat waves was noticed, but no correlation with smog-alert days could be demonstrated.

Massey, Landau, and Deane[4] conducted a study on two synthetic communities within the Los Angeles air basin. These communities, with a combined population of 944,391 persons, were divided into high-pollution and low-pollution areas. The two areas had similar temperatures. The mean number of daily deaths in the low-pollution area was subtracted from

the mean number in the high-pollution area, and the difference was examined by correlation and regression analyses with respect to differences in pollution. The observers were unable to detect any significant correlation between mortality and difference in oxidant, sulfur dioxide, or carbon monoxide content in the ambient air.

Studying specifically the effect of pollutant concentrations on cardiac and respiratory diseases in Los Angeles County, Hechter and Goldsmith[5] compared daily mortality from these causes with fluctuations in oxidant and carbon monoxide concentrations and temperature. The authors removed the statistical effect of season by fitting Fourier curves to the data, which were presumed to be independent of season. When residua from the fitted curves were analyzed, no significant correlations between pollutants and mortality from cardiorespiratory diseases could be found. The authors also applied lags of 1-4 days, but again were unable to demonstrate any significant correlations. It is possible that some additive effect of pollutants on mortality occurred but would not be detected by this analysis.

Another attempt to compare cardiorespiratory deaths with photochemical oxidant pollution was carried out by Mills,[6] who compared seasonally adjusted nursing-home deaths in Los Angeles with measures of photochemical oxidant pollution. He found a suggestive positive association between photochemical oxidant concentration and excess deaths when pollution rose above 390 $\mu g/m^3$ (0.2 ppm). Although heat and seasonal variability were considered, the statistical analyses used make it questionable whether their effect could be suppressed.

A variety of methods have been used to assess a relationship between mortality from chronic illness and photochemical oxidant pollution, but none has been able to demonstrate a clear-cut relationship. The possibility of a

synergistic effect has not been ruled out, although the experience of areas other than the Los Angeles basin with lower concentrations of photochemical oxidant pollution suggests that temperature is an overwhelming factor in these deaths.

Further studies on mortality in relation to photochemical oxidant pollution are needed to delineate differences in susceptibility (especially in children and the elderly), to assess the additive effects of various weather conditions, to examine geographic variations in mortality in relation to pollution, and to determine whether excess mortality is attributable to specific diseases (e.g., emphysema), which may have been overlooked in examinations of total mortality. Populations should also be more carefully categorized to determine whether specific subsamples are at greater risk.

HOSPITAL ADMISSIONS

Several studies have attempted to correlate increased numbers of hospital admissions with variations in photochemical oxidant pollution. The California Department of Public Health study[1] of excess mortality also investigated hospital admissions as a possible health indicator of oxidant pollution. Admissions to Los Angeles County General Hospital in September through December 1954 for childhood asthma, tuberculosis, other respiratory diseases, and all other causes were examined. No significant association with oxidant concentrations was found.

Two other investigators have attempted to relate hospital admissions to increased photochemical oxidant pollutants. Sterling et al.[7,8] studied admissions to Los Angeles hospitals for a 7-month period in

1961. They grouped diseases into "highly relevant," "relevant," and "irrelevant" categories. The "highly relevant" were allergic disorders, inflammatory diseases of the eye, acute upper respiratory infections, influenza, and bronchitis. "Relevant" disorders included diseases of the heart, rheumatic fever, vascular diseases, and all other respiratory diseases. All other diseases were considered "irrelevant." Because both hospital admissions and photochemical oxidant concentrations varied by day of week, corrections for both were introduced into the analysis. Although there was a statistically significant correlation coefficient between hospital admissions for "highly relevant" and "relevant" conditions and photochemical oxidant pollution, the differences were extremely small.

Both "highly relevant" and "relevant" diseases showed some correlation with carbon monoxide and ozone, and nitrogen oxides and particles correlated with "highly relevant" diseases. Even if the relationships claimed by this study are valid, they are small, and confirmation by studies over a long period is needed.

Brant and Hill[9,10] also examined the number of patients who had respiratory and cardiovascular diseases and were admitted to Los Angeles County General Hospital for a 4.5-month period in 1954. These months--generally regarded as the "smog season"--included the highest photochemical oxidant concentrations of the year. Because he accepted into his study group only persons who had resided for at least 3 years within 13 km of downtown Los Angeles and excluded the extremely old and children under 9, the numbers he dealt with were rather small for this brief period. Complex statistical analyses

were used to compare admissions with photochemical oxidant pollution, as measured at a monitoring station not quite 6 km from the hospital. The methods used have been questioned by many competent statisticians, and the claims of a significant correlation between periods of photochemical oxidant pollution and hospital admissions are difficult to accept. Even more difficult to accept is the claim of a positive correlation between high concentrations of photochemical oxidants and hospital admissions 4 weeks later. No reasonable medical explanation can be given for the latter correlation.

Studies to date have demonstrated an extremely weak correlation (if any) between photochemical oxidant pollution and hospital admissions. If such correlations do exist, observations must be carried out over considerably longer periods with adequate provisions for controlling confounding variables, such as meteorologic factors.

ACUTE RESPIRATORY INFECTION

The possibility that chronic exposure to photochemical oxidant pollution alters host resistance and thus increases susceptibility to acute respiratory infection is a challenging one. An opportunity to examine this thesis occurred in 1968 and 1969, when an epidemic of A2/Hong Kong influenza occurred in southern California. Pearlman et al.[11] had the opportunity to observe five southern California communities that varied from high to low oxidant concentrations during this period. Because other pollutants, especially oxides of nitrogen and particles, have shown associations with influenza attack rates, an association with photochemical oxidant pollution was carefully investigated. Pearlman et al. examined the morbidity among 3,500

elementary-school children in five communities. Although these communities showed a definite gradient in chronic oxidant exposure, there was no significant difference in oxidant concentrations immediately before or during the epidemic. Questionnaires to parents of the children followed between November 1968 and January 1969 asked about the presence of influenza-like illness or symptoms. Blood specimens were later obtained from the children whose parents reported upper respiratory symptoms. Titration was performed by hemagglutination inhibition and complement fixation against A2 influenza antigen. Although more children reported illness in low-pollution cities, no statistically significant difference in illness rates could be correlated with exposure to chronic oxidant air pollution. Inasmuch as acute oxidant concentrations were roughly similar in all five communities, it may be that the children did not receive an oxidant dose sufficient to impair host defense mechanisms immediately before or during their exposure to the virus. In statistical manipulation, the investigators were able to minimize other significant covariants, but concluded that "oxidant exposure....did not alter host defense mechanisms enough to cause morbidity differences."

School absenteeism is known to be due in large part to acute respiratory illness, so Wayne and Wehrle[12] followed daily absenteeism rates in schoolchildren in Los Angeles, but were unable to detect any relationship between variations in ambient photochemical oxidant concentrations and school absenteeism.

In a long-term study, Hammer et al. recorded daily symptoms in healthy young student nurses for a period of nearly 3 years (October 1961-June 1964).[13] An average of 61 student nurses in two nursing

schools in Los Angeles completed a daily dairy for 868 days on the incidence of cough, chest discomfort, eye discomfort, and headache--all symptoms that might be expected to accompany an acute respiratory infection. Other recorded information included incidence of gastrointestinal symptoms, physician visits, restricted activity, and menses. Daily maximal hourly concentrations of photochemical oxidants, carbon monoxide, and nitrogen dioxide and daily temperatures were also measured at monitoring stations within 2 miles of the schools. As expected, eye discomfort showed the strongest correlation with photochemical oxidant concentrations, with one-third of the total population reporting the symptom when oxidant concentration reached 0.5 ppm. However, cough and chest discomfort also increased with maximal hourls oxidant concentration. Headache showed a positive association with photochemical oxidant concentration, but the increase in the number reporting the symptom (3-8%) was considerably less than for other symptoms. Temperature and carbon monoxide and nitrogen dioxide concentrations could not explain the associations found. Cigarette-smoking history, allergies, and bias in reporting were supressed as variables in the analysis. It is of particular interest that the authors were able to compute the thresholds of which the prevalence of the symptoms began to increase and found them close to the present U.S. national primary standard for photochemical oxidant, indicating little or no margin of safety. The eye irritation threshold was calculated at approximately 294 $\mu g/m^3$ (0.15 ppm). Because these were all young healthy adults, relatively free from chronic disease, the same effects in elderly persons or in those with chronic heart or lung diseases could be expected to result in greater physiologic embarrassment.

Although the observed threshold for eye discomfort is comparable with reported results of human experimental exposure-chamber studies, it is recognized that current methods for measuring photochemical oxidants primarily measure ozone, which is not known to be an eye irritant. The authors therefore assumed that the measured concentrations of ozone reflected the concentrations of such known eye irritants as peroxyacylnitrate, peroxybenzoylnitrate, formaldehyde, and acrolein--all of which have been shown to be present in Los Angeles basin air. However, the symptoms of cough, chest discomfort, and headache have all been associated with occupational exposure to ozone, so it is possible that ozone itself is the offending agent for these symptoms. It is interesting that the observed daily maximal hourly carbon monoxide concentrations in this study were below those which have been reported to cause headache experimentally. Nevertheless, inasmuch as a significant number of student nurses did report headache associated with increased oxidant concentration, an interaction between carbon monoxide and oxidants cannot be excluded.

AGGRAVATION OF PREEXISTING DISEASE

The best-documented health effect of general urban air pollution is the aggravation of preexisting disease. In the notorious Donora episode and in the many well-documented episodes of excess mortality in London's "smogs," most of those who died had had marked impairment of cardiac or pulmonary reserve. Similarly, the majority of epidemiologic studies demonstrating an association of health impairment with increased air pollution were carried out in areas where the atmosphere was characterized by a particulate-sulfate (and preponderantly chemically reducing) type of pollution. Several investigators have attempted to determine whether similar health effects are produced by exposure to atmospheres that have photochemical oxidants as the characteristic pollutants.

Studies by Motley et al.[14] on 66 volunteers, 46 of whom had pulmonary emphysema, were conducted in a "filtered room" from which oxidants were removed by activated-charcoal filters. Pulmonary function studies demonstrated improvement in emphysematous patients who remained in the room for 40 h or more, if they had entered it on a day when the ambient air in Los Angeles was considered "smoggy" by the investigators and the Los Angeles Air Pollution Control District. Normal subjects showed no change in pulmonary function in the filtered-air chamber, compared with measurements taken when they were breathing ambient "smoggy" air. Emphysematous patients also showed no improvement if they entered the room on a "nonsmoggy" day. The study, although widely cited, is difficult to evaluate, because the number of patients was relatively small. Some improvement was noted

in the emphysematous patients who entered on "smoggy days" in vital capacity, FEV_3, and maximal breathing capacity. The most marked change, however, was a decrease in residual lung volume.

Remmers and Balchum[15] used a specially constructed room at Los Angeles County Hospital with an air-conditioning filter system and filters that could be used at the discretion of the investigator to remove photochemical oxidants and nitrogen oxides from ambient air. Particulate matter could also be partially removed. They carried out studies during September and October 1964 and from March to November 1965. Pulmonary function studies were performed one or more times a day during the approximately 1 week that each patient spent in the room with no filtration and again for a week with filtration. Temperature and humidity were kept constant in both cases. Airway resistance, diffusing capacity, other pulmonary functions, blood oxygen tension, and oxygen consumption were measured when the subjects were at rest and exercising. Examination of the data indicated that airway resistance was affected by increased oxidant concentration over a range of 100 - 450 $\mu g/m^3$ (0.05 - 0.23 ppm). Unfortunately, the data are difficult to interpret, because many of the subjects were cigarette-smokers, the subjects were exposed to ambient air containing many substances in addition to oxidant, and the observed improvement with filtered air may have been caused by the removal of other pollutants, such as aerosols, aldehydes, and particles.

A more elaborate analysis of these data was carried out by Ury and Hexter.[16] It demonstrated that oxygen consumption, in most cases, increased with oxidant concentration. Unfortunately,

the oxidant concentration was measured only twice a day at a station some distance from the site of the subjects' exposure. Oxidant was also measured as ozone, so the concentrations of other irritant oxidant compounds could not be determined.

A much larger group of men was followed by Shoettlin[17] in an attempt to study the long-term effects of community air pollution on persons with and without symptoms of pulmonary disease. These two groups were veterans who lived in the domiciliary unit and in the chronic-disease annex of the Los Angeles Veterans' Administration Center. A group of 528 veterans in the domiciliary unit who had no symptoms of respiratory disease were used as the control population. A group of 326 men were selected on the basis of at least two symptoms of respiratory disease for 2 years or longer. The symptoms included cough, sputum production , shortness of breath, wheezing, and abnormal breath sounds. The two groups were matched by age and smoking history, and the resulting pairs were studied weekly by repeated pulmonary function tests and response to a standardized respiratory-symptom questionnaire. An analysis of variants showed no statistically significant effects of air pollution on incidence or prevalence of respiratory symptoms or on tests of pulmonary function. Nevertheless, the maximal oxidant and oxidant precursor concentrations consistently accounted for more of the variation in frequency of symptoms and clinical signs in the group with pulmonary disease than in the control group. Up to 30% of the variation in symptoms in the group with pulmonary symptoms appeared to be correlated with the maximal oxidant precursor concentrations, whereas no association could be demonstrated in the control group.

ASTHMA

Asthma, a disease featuring sudden and dramatic variations in respiratory symptoms and pulmonary function, is well known to be related to environmental factors in many cases. Many other factors, however, may play a significant role in the precipitation of asthmatic attacks, including meteorologic factors, emotional factors, infection, allergy, and physical activity. It has also been demonstrated that there is a significant ethnic variation in factors associated with acute asthmatic attacks.[18] Nevertheless, between 2 and 5% of the population is subject to asthmatic attacks, so it has been examined as an indicator of health effects of photochemical oxidant pollution.

Schoettlin and Landau[19] carried out one of the earliest studies to determine whether aggravation of asthma could be related to increases in oxidant concentrations. They followed 137 asthmatics in Pasadena with known asthma for at least 5 years. Over at least a 3-month period in late 1956--the usual peak oxidant period of the year--weekly reports were obtained from each patient as to the number of attacks. Of the 3,435 attacks reported by these patients, less than 5% were spontaneously associated by the patient with "smog." Although most reported attacks on days with high enough oxidant concentrations to cause eye irritation and plant damage, the majority of attacks occurred between midnight and 6:00 a.m., whereas peak oxidant concentrations occurred between 10:00 a.m. and 4:00 p.m. This lag, however, would not necessarily rule out a causative role of oxidants in asthmatic attacks. The conclusion of the authors was that only 8 of the 137 patients could be characterized as "smog reactors."

The oxidant concentrations later reported by Renzetti and Marcus[20] were a peak of 0.25 ppm and, for the same period and same station, a maximal hourly concentration of about 0.02 ppm. It is of interest that one-third of the attacks spontaneously associated with "smog" were reported by a single patient. However, in spite of the small number of people who spontaneously associated their attacks with "smog," this study suggests that there are probably some people who do experience asthmatic attacks related to increased concentrations of photochemical oxidants. Further studies to confirm this, sponsored by the EPA, are underway in the Los Angeles air basin.

EFFECTS OF PHOTOCHEMICAL OXIDANTS ON HEALTHY POPULATIONS

Athletic Performance

Wayne et al.[21] related athletic performance of high-school cross-country runners over a 5-year period from 1959 to 1964 to oxidant measurements for the hour of the race and 1, 2, and 3 h before the race. Temperature, relative humidity, wind velocity and direction, and oxides of nitrogen were also considered, but showed no relation to the running times. A "training effect" that might be expected to improve performance on each succeeding race was also considered. A significant relationship, however, was observed between high oxidant concentrations and the percentage of runners whose performance decreased, compared with their performances in the previous home meet. It is of interest that the deterioration in performance of these 21 runners was noted beginning at 130 $\mu g/m^3$. With increasing oxidant concentration, there was a manifest deterioration in team performance

over a range of 60 - 590 $\mu g/m^3$. This deterioration in performance began well below the national photochemical oxidant standard of 160 $\mu g/m^3$ (0.08 ppm). The authors readily admitted that "the observed effects may be more related to the lack of maximal effort due to the increasing discomfort than to decreased physiologic capability." Nevertheless, although they discussed the possibility that decreasing performance might be due to the detrimental effect of discomfort from eye irritation, the data provide convincing evidence that some components of the air that were measured as oxidant had an effect on team performance.

Although no threshold effect was specified by Wayne et al. a later analysis by Hasselblad et al.[22] suggested a threshold estimate of 235 $\mu g/m^3$ (0.012 ppm). A similar study was carried out over a shorter period by Koontz[23] in Seattle, where oxidant concentrations were approximately one-third those in Los Angeles. A decrease in performance of long-distance runners was noted with increasing oxidant concentrations, but these were also associated with marked temperature increases, and it was impossible to separate their relative contributions.

Automobile Accidents

Another association of oxidant concentrations with potentially serious implications is the increase in automobile accidents noted by Ury.[24] He recorded accidents in each daylight hour of each weekday in the "high-smog" 3-month period between August and November for 2 years and found a statistically significant relationship between oxidant

concentrations and the number of automobile accidents. He was unable to demonstrate a similar relationship with nitrogen dioxide. Because the study compared hours with presumably similar traffic density, the likelihood that traffic density accounted for the association is probably small. The data suggest that photochemical oxidant pollution impairs driving performance, either directly by interfering with oxygen transport or utilization or indirectly by causing eye discomfort or respiratory irritation. It is also possible that other pollutants, such as carbon monoxide emitted from the automobile tailpipe, account for some or all of the excess accidents.

Discomfort

Additional evidence of an association of discomfort or more severe health effects with increased oxidant concentrations was published by the Committee on Air Pollution of the Los Angeles County Medical Association.[25] The organization circulated a questionnaire to a sample of every sixteenth physician registered to practice in the County. Although air pollution was not specifically mentioned in the questionnaire, the fact that it was sponsored by an air pollution committee probably suggested to the physicians that air pollution was the principal environmental concern. The physicians were asked how many patients they had advised to move from Los Angeles County for health reasons before December 1960. By extrapolation from these questionnaires, it was estimated that over 10,000 persons had been advised by their physicians to move from the County for health reasons and, for two-thirds of these, air pollution was given as the reason. Because this was a study of attitudes, it cannot be categorically cited as indicative of a definite health effect.

Pulmonary Function

Children have often been considered to be particularly sensitive indicators of environmental challenge and therefore to constitute a group in which adverse health effects of air pollution might be detected at lower concentrations than in adults. Two groups of elementary-school children living in the Los Angeles basin were assessed twice a week by McMillan et al.[26] with a Wright peak-flow meter to measure ventilatory performance. One group of 50 children lived in an area exposed to high oxidant concentrations, and the other group of 28 children lived in a less polluted area. During 11 months of the study, no correlation could be found between acute changes in oxidant concentration and ventilatory performance in either group. Unfortunately, there were significant differences between the two groups, in that the group in the more highly polluted area was ethnically mixed and had an incidence of upper respiratory tract infection 3 times greater than that reported in the other group. Pulmonary function studies, as part of the Community Health Effects Surveillance Studies (CHESS) program, are underway in seven communities with graduated concentrations of photochemical oxidants in the Los Angeles basin. It is hoped that these will yield more definitive results.

A similar study on the relations between pulmonary function and exposure to photochemical oxidant pollution was carried out by Cohen et al.[27] To eliminate the effects of smoking, the study was confined to Seventh Day Adventists who abstain from tobacco. They were divided into two groups: one living in the San Gabriel Valley (a high-oxidant-pollution area), the second living in San Diego, which was then considered to represent a relatively low-pollution area. Each participant was

interviewed by a physician, underwent a battery of pulmonary function tests, and completed a standardized respiratory-disease questionnaire. Daily maximal hourly average oxidant concentrations in the San Gabriel Valley were twice those in San Diego during the time of the study (San Gabriel, about 274 $\mu g/m^3$, or 0.14 ppm; and San Diego, 137 $\mu g/m^3$, or 0.07 ppm). In spite of the marked difference between the oxidant concentrations in the two areas, no significant differences could be found in pulmonary function studies, the prevalence of chronic bronchitis, or the occurrence of respiratory-disease symptoms, as determined by the questionnaire. In this study, the differences may have been due to the absence of cigarette-smoking in both groups; some investigators have suggested that smoking is an additive factor in the effects of ambient air pollution.[28] It must also be noted that, although the peak values of oxidant pollution in the more polluted area were twice those in the less polluted area, the mean yearly averages were essentially the same.

Kagawa and Toyama[29] in Tokyo followed 20 normal 11-year-old schoolchildren once a week from June to December 1972 with a battery of pulmonary function tests. Environmental factors studied included oxidant, ozone, hydrocarbon, nitric oxide, nitrogen dioxide, sulfur dioxide, particles, temperature, and relative humidity. Temperature was found to be the most important environmental factor affecting respiratory tests. The observers noted that pulmonary function tests of the upper airway were more susceptible to increased temperature than those of the lower airway. Although the effect of temperature was the most marked, ozone concentration was significantly

associated with airway resistance and specific airway conductance. Increased ozone concentrations usually occur at the same time as increased temperature, so their relative contributions could not be determined.

Eye Irritation and Lacrimation

Eye irritation and lacrimation are by far the most widespread and common symptoms clearly associated with increased photochemical oxidant pollution. Although photochemical oxidant is customarily measured as ozone, ozone itself is not a primary eye irritant. Thus, the eye irritation associated with increased photochemical oxidants is probably due to some or many of the complex organic oxidants produced in photochemical "smog." Richardson and Middleton[31,32] noted nearly 20 years ago that eye irritation could be expected in a considerable portion of the population when the oxidant concentration reached 200 $\mu g/m^3$.

Renzetti and Gobran[33] carried out a controlled study on two groups of 20 female telephone-company employees working in adjacent rooms for a period of 120 days. Filters to remove eye irritants were switched periodically between the rooms, so that the two groups were alternately exposed to test and control conditions. Subjects were unaware of whether air was filtered or unfiltered at any given time, but they consistently reported eye irritation when the oxidant concentration exceeded 200 $\mu g/m^3$. Eye irritation has also been previously cited as the most commonly reported symptom in the student-nurse study of Hammer et al.[13]

That the problem of eye irritation in urban environments is not confined to Los Angeles is indicated by the studies of McCarroll et al.[34,35] and Mountain et al.[36] on a population in the lower east side of Manhattan. This population of approximately 2,000 persons of all ages representing the major ethnic groups of New York City was followed for a period of 3 years with weekly interviews. Participants were queried about the occurrence of many different diseases, disease symptoms, aggravation of preexisting diseases, and a variety of other health factors. Frequency of reports of new eye irritation increased concomitantly with increases in oxidant concentrations in the neighborhood. Exact oxidant concentrations could not be measured in the study, because of the presence of high concentrations of interfering substances, including sulfur oxides, particles, and carbon monoxide. Substances other than oxidants might have contributed to the eye irritation reported by this population.

For the two most prevalent symptoms related to photochemical oxidant exposure--eye irritation and lacrimation--no method of quantification has been developed. Eye irritation, although undoubtedly real, is a purely subjective response of the subject, and no measurement, other than the complaint itself, has yet been developed. Similarly, a routine objective measure of lacrimation remains to be developed. However, studies on tears have demonstrated that, when a person is experiencing eye irritation, the lysozyme content of the tears is lower than normal.[37] Measuring lysozyme content of tears or the related pH variation appears promising, but more feasibility studies are necessary before the usefulness of the method is known.

A survey of the state of knowledge of eye irritation and lacrimation in response to photochemical oxidant pollution was carried out by Wilson of the Copley International Corporation for the Coordinating Research Council.[38] After reviewing the studies cited above and related toxicologic work, the report concludes: "In many ways, the understanding of eye irritation produced by photochemical smog has not kept pace with the understanding of other smog manifestations. Part of this is no doubt due to the fact that air pollution control agencies are not set up to collect data from human panels but rely exclusively on instruments. If an objective measure were available for measuring eye irritation, and if this measure were sensitive enough to be useful below the threshold of most humans, then a straightforward study could be done on natural smog."

Nevertheless, in spite of the many gaps in our knowledge of the components that produce eye irritation, there is remarkable uniformity in the findings of several epidemiologic studies cited, in the prevalence of the symptoms as oxidant concentrations increase, in the distress that oxidants cause the affected subjects, and in the threshold concentration at which the symptoms appear (0.15-0.2 ppm).

PHOTOCHEMICAL OXIDANT POLLUTION AS A CAUSE OF CHRONIC DISEASE

Several studies attempting to relate aggravation of preexisting chronic disease to increases in photochemical oxidant pollution have been discussed. The possibility has also been raised that photochemical oxidant pollution plays a role in the initiation of some chronic diseases. In part, this possibility was suggested by laboratory studies demonstrating some

radiomimetic properties of ozone. Although ozone may mimic ionizing radiation in some respects, it carries by no means all the carcinogenic implications. Nevertheless, experiments have indicated chromosomal damage to living cells by ozone[39] and to human cells *in vitro* by organic peroxides.[40] Work of Palmer and colleagues[41] has suggested that ozone is a potential cocarcinogen--a finding that carries great implications during a time of increasing rates of human lung cancer.

To assess the possible contribution of photooxidant pollution to lung-cancer mortality, Buell *et al.*[42] carried out a prospective study of lung cancer among 69,160 members of the American Legion--residents of Los Angeles County, the San Francisco Bay area, San Diego County, and all other California counties. This study was carefully adjusted for cigarette-smoking habits, occupation, and duration of residence in the same county. Death certificates for the first 5-year period were checked for mortality from cancer of the lung and from other chronic lung conditions for a total of 336,571 man-years of observation. As would be expected, residents of the urban counties of Los Angeles, the San Francisco Bay area, and San Diego had considerably higher mortality, both from lung cancer and from other cronic pulmonary disease. Even when adjusted for cigarette-smoking, these differences persisted, but they are compatible with other observations on the well-known "urban factor" in increased death rates from lung cancer, other cancers, and other chronic pulmonary disease. When heavy smokers (more than one pack a day) were examined, the relative risk of lung cancer was found to be greater in Los Angeles County than in other areas of California. For nonsmokers, however,

the rate in the San Francisco Bay area and San Diego County was slightly higher than the rate in Los Angeles County. From these data, it was not possible to demonstrate any effect of oxidant pollution on lung-cancer mortality, if one assumes that in the years of observation (1958-1963) oxilant concentrations were higher in Los Angeles. However, because it is well known that the development of overt lung cancer is an indolent process that takes many years, the period of observation may not have been long enough to detect an environmental effect.

When the same observers examined mortality from chronic respiratory diseases other than cancer of the lung, the death rates were found to be somewhat higher in Los Angeles than in the San Francisco Bay area and San Diego County, particularly among persons who had lived for 10 or more years in the same area. It is well known, however, that socioeconomic class is an important factor in mortality from chronic respiratory disease,[43] and no data are available to show such differences among the groups examined in this study.

With standardized respiratory-survey techniques, Deane et al.[44] examined a group of West Coast outdoor telephone-company employees whose work and medical and social status were comparable with those of similar groups examined on the East Coast, in the United Kingdom, and in Japan. They noted that, in the group over 50 years old, respiratory symptoms were more frequent in the Los Angeles and in the San Francisco population than in the other areas studied. Twice as many telephone-company workers in Los Angeles complained of persistent cough and phlegm as in San Francisco. The groups were controlled for smoking habits. Surprisingly,

in spite of the difference in reporting of symptoms, there were no important differences in the results of pulmonary function tests between residents of the two areas in this occupational group. These findings are at variance with those of Hackney,[50] who noted seasonal differences, which were greater in San Francisco than in Los Angeles.

DISCUSSION

The EPA is conducting a major study in the Los Angeles basin on the effects of photochemical oxidants on health. It is a survey of schoolchildren in seven communities representing a gradient of oxidant exposure. In addition to comprehensive environmental monitoring data, specific health characteristics will be followed, including chronic respiratory disease in adults, lower respiratory disease in children, acute respiratory disease in both children and adults, pulmonary function in children, aggravation of asthma, irritation of mucous membranes, and tissue residues of trace metals. Complete data from this study will not be available for another 3 years, but data from the first 2 years may become available sooner.

A second study by the EPA will attempt to correlate the effects of photochemical oxidants and cigarette-smoking in promoting chronic respiratory signs and symptoms in cohorts of adolescents and their families. Pulmonary function tests will be included, and this study should do much to answer the vexing questions of the relationship of chronic pulmonary disease and photochemical air pollution.

The abovementioned studies are being carried out by the EPA as part of the CHESS program. Although some of these studies have produced useful information, others are apparently not fulfilling their objectives. Specifically, these studies are designed entirely in-house by full-time employees of the EPA at Research Triangle Park, North Carolina. The studies would have benefited by the presentation of experimental design and proposals to knowledgeable and experienced scientists outside the federal establishment for comment and criticism. Furthermore, some of the information collected in past studies and much that is now being collected is retained by the EPA to be used ultimately in standard-setting, without first being subjected to the scrutiny of the scientific community outside the federal government. To date, there has been relatively little publication in the usual scientific journals, and most data have been issued by the EPA in the form of monographs; hence, external scientific input, although sought, had little effect on the published scientific product. Because most of these studies have been related to disease or symptom prevalence, before-and-after studies of the effects of approved air quality have been impossible to carry out. This criticism applies not only to the CHESS studies, but to virtually all epidemiologic studies cited in this chapter, inasmuch as adequate environmental monitoring was rarely carried out in studies before the last decade. The epidemiologists and statisticians undertaking the CHESS studies are extremely competent and are supported by well-trained engineers, chemists, and meteorologists. Personnel turnover, however, has inevitably affected the continuity of direction of the program.

In addition to these much-needed studies, others should be designed to seek information on human populations on points already raised by important toxicologic and clinical studies. Although epidemiologists have been unsuccessful in relating infectious diseases to oxidant pollution, Coffin and Garner[45] showed that ozone exposure before or after exposure to pathogenic bacteria strikingly increases mortality in mice. They found that ozone not only affected host resistance by inhibiting clearance rates, but also permitted uncontrolled growth of streptococci. This and much other toxicologic evidence that ozone interferes with host defense mechanisms in animals suggest that a similar phenomenon may go unrecognized in humans.

The evidence that ozone is a potential cocarcinogen[41] has grave implications. If the radiomimetic properties of ozone are confirmed, we may well be dealing with a nonthreshold dose-response situation in which no standard can be considered to represent a completely innocuous concentration of pollutant.[46,47] The recent work of Bates and Hazucha showing a synergistic effect of ozone and sulfur dioxide encourages the belief that pollutants cannot be considered separately when standards are promulgated and that the complex interrelationships among them must be considered. It is important to know whether other phenomena well demonstrated in animals, such as tolerance,[48] also occur in humans. And there are suggestions that some type of cross-protection may occur, whereby exposure to ozone provides some protection against other irritant oxidants.[49] Analogues for all these phenomena should be sought in human populations, and methods should be devised for assessing their significance for human health.

RECOMMENDATIONS

Modification of the method in which the CHESS studies are designed and in which the data are displayed would add considerably to their value. The scientific data collected in all the CHESS studies should be made available through accepted scientific publications to the scientific community as a whole.

The continuation of epidemiologic studies, including those of the CHESS program, is vital to our understanding of the effects of air pollution on health. There is no other way to determine the needed dose-response (exposure-response) relationships between the complex urban atmospheres and specific health effects. No animal or clinical experiment can duplicate the full range of variables to be found in ambient urban air. For this reason, all necessary support should be given to qualified scientists to conduct epidemiologic studies designed to answer these questions. Although such studies are expensive and time-consuming, the data they produce can be produced in no other way and are essential in the development of useful air quality standards.

It is important to know whether such phenomena as tolerance and cross-protection, well demonstrated or suggested in animals, occur in man. Analogues for all these phenomena should be sought in human populations, and methods should be devised for assessing their significance for human health.

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Chapter 11

PLANTS AND MICROORGANISMS

The intent of this chapter is to give a critical review of all research related to the effects of photochemical oxidants, including ozone and the peroxyacylnitrates, on plants and microorganisms.

Injury to vegetation was one of the earliest indicators of photochemical air pollution.[369] Injury was first observed in the Los Angeles area in 1944. Since then, there has been a slow but steady increase in research efforts to understand the effects of these pollutants on vegetation. In the late 1960's and early 1970's, there has been a flood of published information.

In attempting to understand the effects of pollutant-pathogen interactions, investigators looked at the effects of oxidant pollutants on individual microorganisms and on these organisms as they infected host plants. Ozone has been tried as a fumigant to protect stored plants and plant parts against infection by parasitic and saprophytic fungi and bacteria. Research on ozone as a sterilant for water supplies and sewage systems is also related to effects on bacteria. Some studies have simply used bacteria as models for the action of ozone in biologic systems.

The chemical composition of the photochemical oxidant complex is discussed in Chapter 2. The major phytotoxic components are ozone, nitrogen dioxide, and the peroxyacylnitrates. The latter homologous series of compounds includes peroxyacetylnitrate (PAN), peroxypropionylnitrate (PPN), peroxybutyrylnitrate (PBN), peroxyisobutyrylnitrate (PisoBN), and peroxybenzoylnitrate (PBzN). The first four of these are known to be toxic to plants, but only PAN and PPN have been reported in sufficient quantities in ambient air to cause visible

symptoms.[506] The effects of the nitrogen oxides (NO_x) are included in another NAS report.[80] Discussion of the quantitative effects of ozone and PAN will be limited to laboratory and controlled field exposures, because, under ambient conditions, the effects of these compounds are difficult to differentiate. Although ozone is, quantitatively, the primary oxidant component of photochemical air pollution, the term "oxidant" is used to include ozone and PAN in discussing biologic effects under ambient conditions. However, some studies suggest that phytotoxicants in addition to ozone and PAN are present in the photochemical complex.[209] Regardless of the number of phytotoxicants present, ozone is the most important, it has received the greatest amount of study, and its effects are better understood. The major thrust of this chapter covers research with ozone.

Before ozone[225,461] and PAN[496] were identified as specific phytotoxic components of the photochemical complex, researchers used a number of artificial chemical reaction systems to simulate the ambient photochemical oxidant situation. These efforts involved a number of irradiated and non-irradiated reaction systems (unsaturated hydrocarbon-ozone mixtures, unsaturated hydrocarbon-NO_x mixtures, and dilute auto exhaust). Most research before 1960 involved one or more of these reaction systems.[102, 103, 132, 209, 210, 215, 365, 368, 401, 505, 515, 553] This research has been well reviewed and thus is not extensively covered here. Although the work is difficult to correlate with research on individual pollutants or combinations of pollutants, many of our present concepts were first enunciated on the basis of these photochemical oxidant simulation systems. Any researcher concerned with the effects of oxidant pollutants on vegetation or microorganisms should be familiar with this early work.

A number of reviews of varied quality cover general or special effects of photochemical oxidants on vegetation (Table 11-1). Thomas[515] fairly comprehensively covered the available information on the effects of photochemical oxidants on plants. Middleton[365] gave the first comprehensive coverage of the phytotoxic effects of photochemical oxidants in 1961. A number of excellent reviews have appeared since 1961. Rich[453] presented an early review of ozone effects. Dugger and associates[131,132] presented the physiologic and biochemical effects of oxidants on plants. Heck[210] covered factors that influenced the expression of oxidant damage. Heagle[196] discussed oxidant problems with respect to interactions between air pollutants and plant parasites. Three chapters in the air pollution atlas (Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas)[215,235,510] stressed components of the photochemical oxidant complex in terms of symptom development and relative susceptibility of plants. Reinert et al.[447] presented an excellent treatment of interactions between several of the photochemical oxidant pollutants and sulfur dioxide. The American Phytopathological Society[12] published a glossary of terms that should be useful to any research worker. In addition, several recent review articles have discussed the effects of photochemical oxidants or ozone on vegetation.[211,223,505,567] Three recent books on air pollution effects on vegetation cover subjects of concern to anyone interested in the photochemical oxidants.[128a,390,398] There are two criteria documents: one for the Canadian Ministry of the Environment in 1975[318] and the other for the former U. S. National Air Pollution Control Administration in 1970.[401] Both focus on the development of criteria useful for the setting of ambient air quality standards. These two reviews pertain primarily to dose-response effects and attempt to define threshold

Table 11-1

Selected Review Articles - A Subject Listing

Subject	References
General	51, 101, 103, 128a, 175, 211, 222, 223, 251, 317, 351a, 364, 365, 367, 368, 390, 398, 505, 515, 516, 517, 565, 566, 571, 584
Criteria documents	318, 401
Symptoms and susceptibility	215, 235, 475, 510, 515
Photochemical oxidants	103, 215
Ozone	235, 390, 453, 567
PAN	79, 390, 510
Physiologic and biochemical	128a, 131, 132, 390
Effects of various factors	196, 210, 447
Plants as monitors	28, 209
Glossary of terms	12

dose or specific concentration and time combinations that produce specific response measures. Neither document contains sufficient information on microorganisms to enable the development of useful dose-response information. The Canadian review[318] is generally more comprehensive than the U. S. document,[401] in that it presents a threshold dose-response curve for use with ozone and, by implication, with ambient oxidant measurements.

RESPONSES OF VASCULAR PLANTS

Injury to vegetation from photochemical oxidants was first characterized in 1944[369] as a glazing, silvering, or bronzing of the lower leaf surfaces of broadleaved plants. These symptoms were first identified in the Los Angeles area, but were soon recognized over a large segment of southern California and in the San Francisco Bay area.[367] Photochemical oxidants are known to injure plants in most, if not all, major metropolitan areas of the United States, Canada, and Mexico and probably affect vegetation in major metropolitan areas throughout the world.

Ozone is generally recognized as the most important phytotoxicant in the oxidant complex. Early studies were reported in 1864,[300] The phytotoxicity was firmly established in 1914,[285a] and this was confirmed in laboratory studies in 1937.[246] It was first shown as a phytotoxic component (causing grape stipple) of the oxidant complex in 1958[461] and later as the cause of weather fleck of tobacco.[225] Ozone is now known to cause injury to a multitude of broadleaved plants and to explain several types of needle injury in both eastern and western conifer species.

The peroxyacylnitrates were first identified in 1961[496] as the primary cause of the undersurface glazing and bronzing on some broadleaved plants. PAN has since been implicated in numerous pollution episodes in southern California. Its importance in other metropolitan and rural areas in the United States and throughout the world is uncertain, although symptoms have been reported elsewhere.[428] This is because oxidant concentrations in other parts of the world are much lower than those reported in California, and monitoring networks have not looked for PAN or its homologues. The report by Penkett et al.[436] is the first to come from Europe.

Research on photochemical oxidants including ozone was confined primarily to California during the 1950's. By 1959, ozone was known to be an important pollutant in the eastern United States and southern Canada. It is now known as a ubiquitous pollutant and has been widely studied throughout the United States and Canada. By 1972, several other countries had recognized the potential effects of photochemical oxidants on vegetation and initiated research.

We shall stress three areas of methodology that are of special concern for the study of air pollution effects on vegetation: growth of the test organisms, exposure facilities, and instrumentation. First, to determine the effects of air pollutants on many plant species, one must have a good understanding of the best cultural conditions for a given test crop; some results reflect the use of poor test specimens. Second, dynamic air-flow designs are required in chamber construction and exposure conditions must be similar to those which occur in the field. The method of preference would be a dynamic, single-pass system,[218] to avoid reactions of reactive oxidants with materials of chamber construction or emanations from the plants. Several useful chamber

designs have been reported in the literature.[24,204,232,332,592]
For routine experiments, temperature, light, and relative humidity should approach ambient conditions, and nutrient status should be conducive to good growth. Third, one should not assume instrument accuracy without adequate calibration, either initially or later. Most plant scientists have used the Mast oxidant instrument to measure ozone and ambient oxidants. One should be aware of the inherent variability among instruments and routinely calibrate them. Calibration of all instruments should be with the EPA standard 1% neutral potassium iodide method, and the values should be so reported. When this is not done, the actual values may be 50-100% greater than the reported values. Most papers do not state the calibration procedure, if any, and some do not mention the monitoring instrument. Investigators who use the Mast instrument over long periods without an adequate maintenance program may also report erroneous values. Calibration and instrumentation problems are reviewed in Chapter 6. For comparison, ozone at 1 ppm is equivalent to ozone at about 1,960 $\mu g/m^3$.

The foliar response of plants is discussed in terms of the visible or subtle effects on individual plants. Visible effects may be defined as identifiable, pigmented, chlorotic, or necrotic foliar patterns that result from major physiologic disturbances. Subtle effects do not produce visible injury, but may include transitory metabolic disturbances, such as changes in rates of respiration, photosynthesis, transpiration, and enzymatic processes. Subtle effects may be measurable in terms of growth or long-term biochemical changes. Both visible and subtle effects are induced by physiologic and biochemical changes in the plant. Subtle changes may also affect reproductive or genetic systems. If cumulative changes occur within individual

plants as a result of visible or subtle effects, these changes may affect plant populations and communities, and this could have adverse effects on ecosystems.[194,568] Community and ecosystem responses are discussed and quantified in Chapter 12.

In general, the direct response of plants to all oxidant pollutants has been in the foliage. In several cases of long-term exposure (grapefruit after several months of exposure to ambient oxidants) or high ozone concentration over a shorter period (apple,[371] peach[490]), injury has been reported on fruit itself.

Visible Symptoms

Visible symptoms are useful in characterizing the response of vegetation to a variety of stresses, including air pollution.[565] Similar symptoms, induced by different stresses, have been well described and may confuse the diagnosis.[60,227,235] On the basis of studies to date, diagnostic techniques that use indicators other than visible injury (e.g., respiration) are less reliable than the subjective judgment of an experienced observer. Thus, it is important that researchers become familiar with injury symptoms caused by oxidants and those caused by other stresses. This section briefly describes the injury symptoms used for diagnostic purposes.

The terms "acute injury" and "chronic injury" are often confused with "acute exposure" and "chronic exposure." Their historical use and the need for brief descriptive terminology suggest that they are still useful terms. Acute injury may affect only a small part of a given leaf. It is a result of destruction of cell contents and always causes cell death. The necrotic patterns in acute injury may be characteristic of a given oxidant. These

patterns at least demonstrate the presence of a chemical toxicant. Acute injury is usually associated with short exposures (hours) to specific oxidants or pollutant mixtures at concentrations that cause acute injury and usually appears within 24 h after exposure. Chronic injury, whether mild or severe, is usually associated with long-term or intermittent exposures to low concentrations of oxidants that do not produce acute injury. Normal cellular activity is disrupted, and the chlorosis or other color or pigment change that follows may eventually cause cell death. Chronic oxidant injury patterns are generally not characteristic and may be confused with symptoms caused by normal senescence, biotic diseases, insects, nutritional disorders, or other environmental stresses. These patterns may appear as early leaf senescence with or without leaf abscission. Repeated short-term exposures to ozone may cause physiologic changes that are responsible for chronic symptoms. However, such exposures may produce small but additive amounts of acute injury that are mistakenly referred to as chronic injury.

Before the identification of ozone and PAN as two major phytotoxic oxidants in photochemical air pollution, simulated "smog" (a variety of chemical mixtures) and ambient pollution were the test atmospheres used. The most complete morphologic and developmental studies were conducted during this time by Bobrov (Glater) and associates.[35-38, 60, 179] These were classic studies that covered a range of plant species, including table beet, annual bluegrass, oat, and tobacco. From these early studies has come our basic understanding of microscopic changes and developmental patterns. These workers first suggested that membrane injury may have led to the initial effects on the chloroplasts. They also defined developmental patterns and showed that leaves near maximal expansion were more sensitive than

young leaves (or leaf tissue) or older leaves. They suggested that maximal sensitivity was related to stomatal function, volume of intercellular spaces, and the extent of suberization of mesophyll cells. Other work has since substantiated their studies.[148, 361, 524, 529, 542]

Bystrum et al.[60] first described morphologic changes in leaf surface waxes of table beet exposed to photochemical oxidants; these changes were different from those associated with aphid feeding. Comparison of oxidant injury with that produced by insects has since received attention from Hibben,[227] who found that ozone injury to the leaves of four tree species produced smaller flecks, randomly spaced and darker than fleck injury along veins induced by a mesophyll-feeding leafhopper.

Although ozone and PAN are considered the two primary phytotoxic oxidants in the photochemical complex, the specific response of plants to many simulated atmospheres suggests the existence of other phytotoxic oxidants.[209] The symptoms associated with many of these reactant mixtures are closely related to those caused by ozone and PAN.[208, 215] In some tests, the mixtures used would not have produced either ozone or PAN. In other cases, leaf age or the pattern of injury on sensitive test plants suggested one or more pollutants other than ozone or PAN. Field injury symptoms often resemble those reported for ozone or PAN, but the response pattern is sufficiently different that accurate diagnosis is difficult. Brennan et al.[50] correlated development of oxidant symptoms with aldehyde concentrations in New Jersey and suggested that aldehyde may be a major phytotoxic component of the photochemical oxidant complex. The symptoms were probably not responses to the aldehyde, but rather to some compound or group of compounds present under the same conditions as the aldehyde.[237a]

The most complete description of ozone injury symptoms is found in the Atlas.[235] However, several of the review articles, including the two criteria documents,[318,401] have injury descriptions. A concise description is found in Heck and Brandt.[211]

The classic ozone symptoms on angiosperms are the upper surface fleck of tobacco[225] and stipple of grape.[461] These classic symptoms were described in plants with differentiated mesophyll and are especially significant, because they were identified with palisade cells, and not with spongy cells.

The two classic symptoms are still more widely associated with the response of dicotyledonous plants to ozone than are other symptoms. Many plants (e.g., pinto bean, cucumber, tomato, soybean, and sycamore) may have the entire upper surface covered with a bleached appearance as a result of ozone exposure, with no observable injury on the lower surface. On closer examination, the bleached area is seen to be made up of many small groups of palisade cells that are dead and contain no pigment. In other plants or under different conditions, the palisade cells may accumulate dark alkaloid pigments (stipple) coincidentally with cell death. After exposure to higher ozone concentrations or after longer periods of exposure, injury extends to the spongy cells, producing bifacial necrosis. Plants exposed to a high concentration of ozone or to a high concentration of ambient pollution during a pollution episode usually develop dark watersoaked areas in the leaf within a few hours. Leaves may show partial recovery, or these areas may form light-tan bifacial necrotic lesions within 24-48 h. Individual lesions may be small, but groups of them can extend and affect a considerable portion of the leaf.

In monocotyledonous plants (grasses and cereals) and some others, there is no division of mesophyll tissue, and injury normally appears as a bifacial fleck.[35] Some plants, after extended exposure to low concentrations of pollution (either continuously or intermittently), produce chlorotic patterns that may be distinctive of oxidant pollution or similar to symptoms of normal senescence. The early senescence seen in some plants may be a result of long-term exposure to ambient oxidants.

The foregoing discussion is not descriptive of effects noted in coniferous trees, such as pine. It is worth discussing two classic oxidant (ozone) syndromes of pine--one eastern, the other western. Ozone is the probable cause of emergence tipburn in white pine (white pine needle dieback).[23] The injury is characterized as a tip dieback of newly elongating needles and occurs throughout the range of eastern white pine. Affected trees are found at random in a stand, and symptoms develop in discrete episodes in successive years. Primary roots of affected trees often die after repeated needle injury. Costonis and Sinclair[85] reported silvery or chlorotic flecks, chlorotic mottling, and tip necrosis of needles as results of ozone exposure. Ozone may be associated with semimature-tissue needle blight (SNB),[314] but Linzon[315] found that both SNB-sensitive and SNB-tolerant white pine were first injured with ozone at about 0.6 ppm for 2 h, and the symptoms were not like SNB.[315] Linzon found that ozone injury began in young tissue with newly functional stomates, whereas in SNB[313] the necrosis begins in semimature leaf tissue and then spreads through the older needle tissue toward the tip. Needle injury resulting in a disease called "chlorotic decline" of ponderosa pine was first noticed in 1953 and was related to oxidant air pollution by 1961.[426] The chlorotic decline was characterized by a progressive reduction in terminal

and diameter growth, retention of only the current season's needles, reduction in number and size of these needles, yellow mottling of the needles, deterioration of the primary roots, and eventual death of the tree. The chlorotic decline was not associated with stresses other than ozone.[425] These symptoms were reproduced by exposing ponderosa pine to ozone at 0.5 ppm for 9 h/day for 9-18 days.[380] Similar symptoms were noted by Richards et al.,[462] who called the disease "ozone needle mottle of pine." Evans and Miller[149,150,151] and Miller and Evans[374] have conducted histologic investigations on injury development in ponderosa pine. They found that chlorotic needle mottle caused by ozone or ambient oxidants was easily distinguished from "winter fleck."[374] A comparison of needle anatomy in four pines of differing sensitivity suggested that sensitivity was related to the number of mesophyll cells per stomate.[150] They also reported an accumulation of chloroplasts and carbohydrate stain in peripheral portions of mesophyll cells of ponderosa needles before development of visible injury that was not related to stomatal presence.[149] These changes were not shown sufficiently to be specific for ozone effects.[151]

Injury from PAN was first described as smog injury by Middleton et al.[369] on spinach, garden beet, romaine lettuce, and chard. They reported a collapse of spongy mesophyll tissue with the development of large air pockets, especially near the stomates. The air spaces gave the leaf the appearance of a glaze and were responsible for this classic PAN symptom. Most of the descriptive work reported by Bobrov and associates[35-38,60,179] from simulated atmospheres and ambient exposures resulted from PAN injury. The classic injury pattern for PAN is glaze followed by a bronzing of the lower leaf surface. Young expanding leaves are normally more

sensitive to PAN than more mature leaves. Physiologic maturity is important, as shown by the banding appearance on many susceptible leaves that do not mature uniformly. If the whole leaf has a similar physiologic age, it may be uniformly injured (e.g., pinto bean). In case of severe injury, bifacial necrosis can occur. Early senescence and leaf abscission are often associated with PAN injury. The most complete description of injury is that by Taylor and MacLean.[510] Symptoms associated with PPN and PBN are similar to those for PAN.

In several cases, symptoms have been associated with dose.[79, 506, 566] At a high dose (0.5 - 1.0 ppm for 0.5 h), sensitive plants show complete collapse. As the dose is reduced, the plants may show necrotic banding, then bronzing and glazing with slight collapse. At 0.1 ppm, chlorosis is the major symptom. In some unpublished work,[79] an upper-surface light-yellow to white stippling has been described on alfalfa after exposure to PAN at 0.02 - 0.1 ppm for 2-6 h. The injury was intercostal on the tip of young leaves and on the base of old leaves. Drummond[128] reported a necrotic fleck and an upper-surface stipple on petunia after a 1-h exposure to PAN at 0.15 ppm.

Physiologic and Biochemical Effects: Mechanism of Action

Many reports on the effects of ozone and PAN on physiologic processes (net photosynthesis, stomatal response, and water relations) and on metabolic activity (including *in vivo* and *in vitro* studies of individual enzymes, enzyme systems, metabolic pathways, metabolic pool relationships, cell organelles, and plant tissue studies) have appeared since 1964.

Stomata are the principal entry sites for ozone and PAN into plant leaves, and stomatal closure effectively protects the plant from injury.[347] Several studies suggest that oxidants may cause stomatal closure.[259, 289, 326, 457]

Stomatal closure was associated with a genetic factor in onion wherein the stomata of sensitive plants did not close.[142] The effect of ozone and PAN on stomatal opening depends on many interacting factors; those representing water stress appear to be the most important. Dean[116] related stomatal density to the difference in sensitivity between two tobacco cultivars. Evans and Ting[153] found that maximal sensitivity of bean primary leaves was not associated with changes in stomatal number or leaf resistance. Ozone exposure caused a decrease in relative water content, but no change in resistance. Bean leaf sensitivity seemed more a function of internal activities.

Isolated enzymes and enzyme systems are affected by exposure to PAN and ozone. It is now recognized that strong oxidants interfere with various oxidative reactions within plant systems. Metabolic pools, including nitrogen and carbohydrate, appear to have some controlling influence on the response of plants to these pollutants. Sulfhydryl groups are primary targets of oxidant pollutants and may be a key to understanding the mechanism of action. Unsaturated lipid components of some membranes are sites of early action of both ozone and PAN.

Ozone. Ozone is the most nearly ubiquitous of the photochemical oxidants, at least in concentrations that cause measureable effects. It has been studied more extensively than the other oxidants.

• Physiologic effects: The physiologic effects of ozone depend on its entry into the internal leaf spaces through the stomata. If the plant

is resistant to ozone even when stomata remain open, mechanisms of resistance, other than stomatal closure, must be operative. The physiologic effects measureable with the intact tissue include effects on respiration and photosynthesis.

Todd[551] reported that the respiration of pinto bean leaves was stimulated by exposure to ozone (at 4 ppm for 40 min). The first measurements were 4 h after the ozone exposure. The respiration rate later declined to the control value. In all cases, increased respiration correlated well with visible injury. Macdowall[321] confirmed these results, but made an additional observation: during the first hour after ozone exposure (at 0.7 ppm for 1 h), and before visible symptoms appeared, respiration was inhibited. The increase in respiration took place only later, when visible symptoms appeared. Dugger and Palmer[129] reported an increase in respiration in lemon leaf tissue after 5 days of exposure to ozone at 0.15-0.25 ppm for 8 h/day. They reported no morphologic changes at that time. Anderson and Taylor[11] found that ozone induced carbon dioxide evolution in tobacco callus tissue. The threshold for evolution was about 0.1 ppm for 2 h in the sensitive Bel W3. The ozone concentration required for maximal carbon dioxide evolution was about twice as much in the more resistant cultivar. Formation of roots decreased sensitivity.

Todd[551] and Todd and Probst[554] also measured the effects of ozone (at 4 ppm for 40 min) on photosynthesis and found that development of symptoms was associated with inhibition of carbon dioxide fixation. This effect was also confirmed by Macdowall,[321] who reported that the inhibition of photosynthesis was greater than that which could be accounted

for by chlorophyll destruction. Hill and Littlefield[234] associated decreased net photosynthesis caused by ozone (at 0.06 ppm for 1 h) with both stomatal opening and rates of transpiration. These studies have generally shown that net photosynthesis can decrease without visible injury.

Pell and Brennan[431] found an initial decrease in net photosynthesis and an increase in total adenylate concentration after a 3-h exposure to an injurious concentration of ozone. Net photosynthesis returned to normal within 24 h. Respiration was usually not immediately stimulated, but it was within 24 h. The authors concluded that the stimulation of respiration was a consequence of cellular injury, whereas the changes in photosynthesis and adenylate content were early events leading to the appearance of ozone injury.

The photosynthetic rate is an important indicator of vigor in ponderosa pine. A reduction in this rate may occur at the threshold dose of ozone without visible symptoms. Miller et al.[379] found that a daily 9-h exposure to ozone at 0.15 ppm reduced apparent photosynthetic rates by 10% after 30 days, without typical ozone symptoms. Botkin et al.[40] found that a threshold ozone dose for suppression of net photosynthesis in eastern white pine was a 4-h exposure to 0.50 ppm.

Ozone causes both quantitative and qualitative changes in carbon dioxide fixation patterns. Wilkinson and Barnes,[590] using carbon dioxide-^{14}C, found a reduction in radioactivity in soluble sugars and increases in free amino acids and sugar phosphates in white pine after a 10-min exposure to ozone at 0.10 ppm. Miller[372] observed a decrease in carbon dioxide-^{14}C fixation in ponderosa pines that correlated with loss of chlorophyll, after exposure to ozone at 0.30 - 0.35 ppm. The Hill reaction rates of chloroplasts isolated from healthy and ozone-injured

ponderosa pine indicated that both light and dark reactions of the chloroplasts from ozone-injured plants were depressed. Barnes[15] found depressed photosynthesis and stimulated respiration in seedlings of four pine species of the southeastern United States after exposure to ozone at 0.15 ppm.

It is commonly observed that leaves exposed to ozone develop dark green areas--sometimes referred to as a "waterlogging" effect. If symptoms develop, they will be in these "waterlogged" areas, but symptoms are not always a consequence of the "waterlogging." In other words, there is sometimes recovery from this initial effect. The effect is due to leakage of cell contents into the intercellular spaces and, to some extent, is reversible. Many experiments with radioactive inorganic ions and organic substances have confirmed that plant cell permeability is affected by exposure to ozone.[154] Perchorowicz and Ting[437] found that, immediately after ozone exposure (at 0.4 ppm for 1 h), there was no uptake of glucose-^{14}C, but the uptake slowly increased for a period of hours. However, the metabolism of the glucose into various products (organic acids, lipids, carbon dioxide) was not changed by the ozone exposure.

Although it is widely believed that the effects of ozone on cell permeability are the basis of ozone toxicity,[533] the chemical basis of the effects on membranes is still arguable. It is still not clear whether the first effect of ozone is on the protein or lipid components of the cell.

Dugger et al.[133] noted that the susceptibility of pinto bean primary leaves to ozone (at 0.3 - 0.7 ppm for 25-90 min) is maximal at a leaf age of 10 days, when the starch content is high and that of soluble carbohydrate is low. They found that "starvation" of the leaves, by placing plants in the dark for 48-72 h, depleted the carbohydrates and conferred resistance.

Susceptibility was regained by supplying sucrose through the petiole. However, susceptible leaves could be made resistant by supplying more sucrose through the petiole. They concluded that the leaf was resistant when the soluble-sugar content was either above or below the range of 1.0 - 4.0 mg/g of fresh weight. These results were confirmed by Lee,[302] who concluded that the protective action of high sugar content could be partly, but not completely, explained by causing closure of stomata (ozone at 0.8 ppm for 5 h). Barnes and Berry[16] reported seasonal changes in the soluble sugars of white pine that may relate low sugar contents to a prolonged sensitivity to ozone. Hanson and Stewart[189] found that several species exposed to noninjurious concentrations of ozone retained higher starch concentrations in their tissue after 12-36 h of darkness.

Macdowall[320] found that tobacco leaves were most susceptible to injury by ozone (at 0.035 ppm for 5 h) just after full leaf expansion. This point corresponded to the beginning of the decline in protein content. Lee[303] modified the nitrogen content of tobacco leaves by supplying urea and found a positive correlation of injury caused by ozone (at 1 ppm for 5 h) with nonprotein nitrogen, but not with protein. This result is in contrast with that of Ting and Mukerji,[531] who found that, in cotton leaves (in which the period of maximal susceptibility was at about 75% of full leaf expansion), the amino acid pool was low at the time of maximal susceptibility. However, ozone treatment (0.7 ppm for 1 h) increased the free amino acid pool.

At the moment, it is impossible to give a rational explanation of the relationship between ozone injury and carbohydrate and nitrogen composition of the leaf. The compounds measured (protein and carbohydrate) may be only distantly related to metabolites that confer resistance or susceptibility.

It seems reasonable that susceptibility and resistance of different plant species and different varieties within a species should depend on concentrations of endogenous antioxidants. The results of such studies do not give a clear picture: Hanson et al.[191] concluded that the range of susceptibility in petunia varieties depended on the ascorbic acid concentration, but Menser[353] found that the ascorbic acid content of tobacco varieties was not related to ozone susceptibility. Ozone resistance of plants can be conferred by application of antioxidants. In the case of white bean, ascorbic acid, cysteine, glutathione, and nickel N-dibutyldithiocarbamate were effective.[105, 170] Field studies with tomato and tobacco showed that a number of antioxidants were effective protectors: manganous 1,2-naphthoquinone-2-oxime, cobaltous 8-quinolate, nickel di-N-butyldithiocarbamate, N-isopropyl-N-phenyl-p-phenylenediamine.[454] These preventives do not yet present an economically feasible practice for agriculture.

- Biochemical effects: It is usual to find some changes in the chemical composition of plant tissue after exposure to ozone. One cannot be certain whether the changes are associated with early reactions to ozone or are merely delayed consequences of cell injury.

A number of histologic and histochemical changes in current-year needles of ponderosa pine were detected after five to seven daily exposures to ozone at 0.45 ppm for 12 h each day.[149] Chloroplasts and carbohydrate stain accumulated in the peripheral portions of mesophyll cells; concurrently, the homogenous distribution of proteins and nucleic acids was disrupted, and acid phosphatase activity increased. Cell wall destruction occurred in mesophyll cells after appreciable intracellular damage.

Howell[251a, 252] and Howell and Kremer[255] found phenol accumulation associated with the exposure of plants to ozone. Menser and Chaplin[356] also reported an increase in the phenol content of tobacco leaves injured by ambient oxidants. Hoffman[243] reported as much as an 80% increase in nicotine in leaves exposed to ozone at 0.24 ppm for 4 h. No increase was found after exposure to 0.06 or 0.12 ppm. Keen and Taylor[271] found accumulation of several isoflavonoid compounds in soybean (Harosay 63) exposed to ozone. The presence of these compounds suggests a defense mechanism against ozone that is similar to that for disease resistance.

Tomlinson and Rich[556] reported an increase in γ-aminobutyric acid in bean leaves after exposure to ozone (at 1 ppm for 30 min). In a more comprehensive study, Ting and Mukerji[531] repeated this finding in cotton leaves, but found increases in many other amino acids. The only decreases found were in phosphoserine, phosphoethanolamine, ethanolamine, phenylalanine, and alanine. There are discrepancies in the literature regarding the time after ozone treatment before detection of changes in amino acid composition. Ting and Mukerji[531] found the increase only after 24 h, whereas Tingey et al.[543] reported the change to be immediate. Similar differences concern soluble protein: Tingey et al.[543] noted a rise after 24 h, whereas Craker[92, 94] found a decline in protein content. As far as specific proteins are concerned, both Tingey et al.[543] and Leffler and Cherry[305] found a decrease in nitrate reductase; Yamaga et al.[595] reported a decrease in carbohydrase. Dass and Weaver[106] found significant increases in peroxidase and cellulase, but no change in lactate dehydrogenase; Curtis and Howell[97] reported an increase in peroxidase isoenzyme activity. Tingey has also reported an increase in the G-6-PD activity.[534] It is

possible to fit the latter change into a rational scheme for the effects of ozone, but "rational" is not synonymous with "right." Chang and Heggestad[72] found that ozone at 0.35 ppm for 50-80 min impaired the activity of photosystem II in spinach and reduced the β-carotene of the chloroplasts. Coulson and Heath,[87] using isolated chloroplasts, found an inhibition of both photosystems and no penetration of the grana. They suggested that in the intact plant ozone affects only the first membrane contacted.

Chang[69,70,71] has made observations on the polysomes of pinto bean leaves exposed to ozone (at 0.35 ppm for 20-50 min). He found that the chloroplast polysomes were more susceptible to degradation than were the cytoplasmic ribosomes. The sulfhydryl content of the chloroplast ribosomes was also much more susceptible to oxidation than was that of the cytoplasmic ribosomes. Finally, it was found that the effects of ozone on ribosome composition could be reproduced by p-mercuricbenzoate. Chang's results imply that either ozone itself or a product of ozone oxidation passes from the cytoplasmic membrane to the interior of the chloroplast before having its effect. These results connect with a number of papers on the oxidation of sulfhydryl compounds by ozone. Tomlinson and Rich[557] have reported decreases in leaf sulfhydryl groups after ozone exposure (at 1 ppm for 30-60 min). A number of investigators have found that reagents that react with sulfhydryl groups can reproduce the symptoms of ozone damage. Dass and Weaver[105] reported this effect in white bean. Tomlinson and Rich[557] reported the same effect in tobacco, and Rich and Tomlinson[455] found that conidiophores of Alternaria solani became more susceptible to ozone if they were pretreated with iodoacetamide.

It appears that effects on sulfhydryl content may be quite early events in the toxic reactions initiated by ozone. One should remember, however, that symptoms of ozone and PAN injury are quite different, and one cannot explain the effects of both pollutants as sulfhydryl oxidizers.

Tomlinson and Rich[558,559] have also considered the reaction of ozone (at 1 ppm for 30 min) with lipid components of the leaves. They found that a change in the fatty acid composition as a result of ozone treatment was an increase in the saturated fatty acid content. It was concluded that malonaldehyde (an index of lipid peroxidation) was formed only in the later stages of ozone damage. Frederick and Heath[168] reported that the production of malonaldehyde is correlated with loss of viability of Chlorella sorokiana exposed to ozone (at 1.8 ppm for 5-20 min), but with a lag after the initiation of ozone exposure. Swanson et al.,[499] in agreement with Tomlinson and Rich, found only small changes in the fatty acid composition of tissue exposed to ozone (at 0.3 ppm for 2 h). It is not possible to conclude from the available data that oxidation of lipid is the primary effect of ozone exposure. Tomlinson and Rich[560] have examined the effects of ozone on the complex lipids of plants. Plants were exposed to ozone at 0.25 ppm until the first symptoms were seen (2.5-3 h), and leaf solubles were extracted. It was found that there were a decrease in free sterol and increases in both sterol glucoside and acylated sterol glucoside.

Tingey et al.[543] found an increase in reducing sugars soon after the exposure of soybean plants to ozone. In experiments with ponderosa pine, Miller et al.[379] found that the polysaccharide content of both current and 1-year needles, treated for 33 days with ozone at 0.30 ppm, was lowered

by 40%, but there was a slight increase of soluble sugars. No relationship with ascorbic acid was found. Barnes[14] reported studies with five pine species exposed to ozone at 0.05 ppm for 5-22 weeks. There were significant increases in total soluble carbohydrates, reducing sugars, and ascorbic acid. At 0.15 ppm, the effect on ascorbic acid was not observed. These results do not lead one to believe that ascorbic acid plays an important role in ozone resistance.

Nobel and Wang[405] reported that the permeability of the outer membranes of chloroplasts was increased by exposure to ozone (at 30 ppm for 5 min). They hypothesized that the effect was lipid oxidation. Freebairn[169] showed that ozone inhibited respiratory activities of isolated mitochondria, and Lee[304] found that the effect of ozone on oxidative phosphorylation was greater than on oxygen uptake. Mudd et al.[395] reported that the metabolism of UDP-galactose by isolated chloroplasts was inhibited by ozone (at 1,000 ppm for 2 min). There were differential affects on the synthesis of various galactolipids, the synthesis of monogalactosyldiglyceride being relatively resistant. This differential effect of ozone could be reproduced by chemicals that react with sulfhydryl groups.

There is some information concerning the reaction of ozone with chemicals under aqueous conditions. The information available suggests that double-bond cleavage takes place, just as it does under nonaqueous conditions, except that ozonides are not formed. Instead, the zwitterionic intermediate reacts with water, producing an aldehyde and hydrogen peroxide. In addition to double-bond cleavage, a number of other oxidations are possible. Mudd et al.[394] showed that the susceptibility of amino acids is in the order cysteine, tryptophan, methionine, histidine, tyrosine,

phenylalanine, and cystine (ozone at 2,000 ppm for 2-10 min). All other amino acids are resistant. Todd[550] tested the susceptibility of catalase, peroxidase, urease, and papain to ozone. Papain was the most susceptible; this result is consistent with the knowledge that a sulfhydryl group is necessary for its activity. The inactivation of avidin[394] and lysozyme[307] can be attributed to the oxidation of tryptophan residues, although, in the case of lysozyme, methionine is also partially oxidized. Enzyme inactivation caused by ozone reaction with histidine has been indicated in the case of pancreatic ribonuclease.[394] The reaction of nicotinamide derivatives with ozone has been studied by Mudd et al.[392] (ozone at 0.4 - 800 ppm for 1-100 min). The reduced forms are quite susceptible, and the oxidized forms quite resistant. The adenine moiety is quite resistant. When NADH is oxidized by ozone, the 5,6 double bond is broken, so the coenzyme is no longer biologically useful.

Studies of the reaction of ozone with simplified lipid systems have shown that malonaldehyde can be produced by direct ozonolysis.[393] The use of malonaldehyde assay as an index of lipid peroxidation is therefore invalid in ozone studies. Liposomes formed from egg lecithin and prepared in aqueous media were quite resistant to ozone, but the contribution of polyconcentric spheres to this resistance has not been fully assessed.[395] However, the bilayer configuration, with the susceptible unsaturated fatty acids shielded from ozone by the hydrophilic areas of the molecule, may be resistant. In hexane, where the fatty acid moieties are exposed, ozone reacts stoichiometrically with the double bonds. The experiments with aqueous suspensions of phosphatidylcholine gave no evidence of the formation of lipid peroxides,[528] nor did experiments with films of fatty acids exposed to ozone.[465]

• Chemical basis of toxicity: There is only one comprehensive theory for the action of ozone on biologic organisms--the theory of Chow and Tappel[73] that the initial event is the formation of lipid peroxide and that successive events are an attempt to detoxify this product. The theory was developed from experiments with animals that show that exposure to ozone increases malonaldehyde, glutathione peroxidase, glutathione reductase, and G-6-PD.

$$\text{lipid} \xrightarrow{O_3} \text{lipid peroxide (ROOH)},$$

$$\text{ROOH} + 2\text{GSH} \xrightarrow{\text{GSH peroxidase}} \text{ROH} + \text{GSSG},$$

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GSSG reductase}} 2\text{GSH} + \text{NADP}^+,$$

$$\text{NADP}^+ + \text{G-6-P} \xrightarrow{\text{G6P dehydrogenase}} \text{6PG} + \text{NADPH} + \text{H}^+.$$

This theory is questionable on a number of points: there is no direct evidence of formation of lipid peroxide, GSH can be oxidized directly by ozone or used to reduce hydrogen peroxide produced from ozone reactions, and the increased activity of G6P dehydrogenase may be a response to the oxidation of NADPH.

Peroxyacylnitrates. The peroxyacylnitrates are the most phytotoxic of the known photochemical oxidants. The toxicity of the known homologues is inversely correlated with molecular weight. PAN is the best known and has been studied most, because of its usual presence in photochemical atmospheres.

• Physiologic effects: Leaf age and illumination have been studied as physiologic and physical factors that affect the response of plants to PAN.[135] Leaves of most plants are most susceptible when very young. In primary leaves of pinto bean, the period of maximal susceptibility to PAN precedes that for ozone by several days (about 5-7 days from seed for PAN, as opposed to 9-13 days for ozone). The example of tomato is particularly striking, inasmuch as the terminal leaflet of the compound leaf is the oldest tissue. Thus, the youngest susceptible leaf is affected in the terminal leaflet; but, as the leaves become older, the terminal leaflet is resistant and the lateral leaflets are injured.[510]

There is no reason to believe that stomatal opening is different in leaves of different ages, so the entry of PAN into the intercellular spaces of the leaf is probably the same as the entry of ozone. One can hypothesize that the chemical composition of the leaf is such that resistance is conferred at particular physiologic states. Such a hypothesis would lead to a search for compounds that combat the oxidizing and acylating properties of PAN.

There appears to be an absolute requirement for illumination before, during, and after fumigation with PAN (1 ppm for 30 min), if symptoms are to be observed.[513] If the prefumigation light period is followed by 15 min of darkness before a 30-min exposure to PAN in the light, no symptoms are observed. Thus, at this time, a short dark period before PAN exposure prevents damage. However, the dark period after fumigation must be 1-2 h long, if damage is to be avoided. The light period after fumigation must be 3 h long, if damage is to be maximal.[506] These effects of illumination are not understood.

The action spectrum for the light requirement[134] shows maximal quantum responsivity at 420 and 480 nm and low responsivity at wavelengths characteristic of chlorophyll (PAN at 4 ppm for 15 min).[134] The action spectrum suggests participation of either carotenoids or flavins. It should be emphasized that the action spectrum for illumination was determined only during fumigation. It is possible that a different photoreceptor is involved in the periods before and after exposure. Photosynthesis of intact cells of Chlamydomonas reinhardi[180] was inhibited by PAN (at 125 ppm for 5 min). Photosynthesis recovered after the exposure, but the recovery varied with the light quality used during the exposure. Recovery was less when the irradiation was at 450 nm; this is roughly consistent with the work on the action spectrum in pinto bean leaves.[134]

Although the quantum responsivity is low at 660-700 nm, effects of irradiance at these wavelengths are noticeable. In a comparison of the effects of light at 660 and 700 nm, injury was greatest when plants were irradiated at 660 nm during exposure and lowest when they were exposed either in darkness or at 700 nm (PAN at 1 ppm for 15 min).[130] The most striking observation was that simultaneous exposure to light at 660 and 700 nm resulted in little injury. These results were interpreted as effects on photosynthetic systems I (activated at 700 nm) and II (activated at 660 nm), but effects of these wavelengths on phytochrome transformation should not be overlooked:

$$P_r \underset{700\ nm}{\overset{660\ nm}{\rightleftharpoons}} P_{fr}$$

where P_r is the red form of phytochrome and P_{fr} is the far-red form.

It is noticeable that the action spectrum for the PAN effect is similar to that for phytochrome and for some physiologic responses mediated by phytochrome.[59, 383] Interaction of PAN with the phytochrome system seems worthy of systematic study. The light effects may also be related to the sulfhydryl content of plants, inasmuch as illumination increases and exposure to PAN (at 1 ppm for 15 min) lowers the sulfhydryl content.[130]

It has been suggested that sulfhydryl compounds are susceptible to reaction with PAN (by oxidation or acetylation), and thus that conditions that increase the sulfhydryl content will increase susceptibility. This explanation for PAN injury is consistent with the finding that inhibition of photophosphorylation by N-ethylmaleimide, a reagent that reacts with sulfhydryl radicals, is most effective when chloroplasts are treated in the light.[350a] In the Calvin and Hatch-Slack pathways of carbon dioxide fixation in photosynthesis, some enzymes are activated by light (e.g., ribulose phosphate kinase),[59] and some are inactivated by light (e.g., glucose-6-phosphate dehydrogenase or G-6-PD).[9] In both cases, the effect of light can be replicated with sulfhydryl compounds, such as dithiothreitol. The reaction of PAN with the sulfhydryl forms of these enzymes can easily be visualized as upsetting the regulation of carbon dioxide fixation. These considerations are consistent with the effects of PAN on various aspects of photosynthesis (adenosine triphosphate, ATP, formation; nicotinamide adenine dinucleotide phosphate, NADP, reduction; and carbon dioxide fixation) and with the fact that the earliest injury found by the electron microscope was in the stroma of the chloroplasts (PAN at 1 ppm for 30 min).[525]

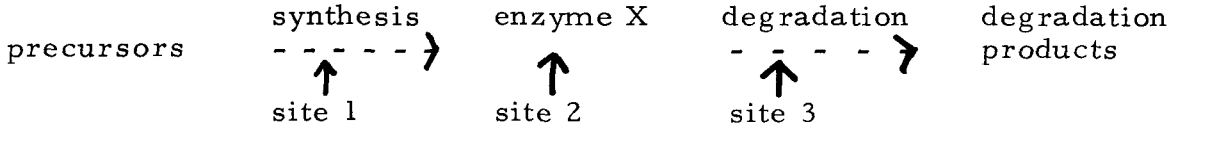
- Biochemical effects: Several enzymes that use nicotinamide cofactors were found to be inhibited by PAN (at 125 ppm for 1 min)[387] in

in vitro studies. These enzymes were most susceptible in the absence of substrates. In some cases, an enzyme was protected by the nicotinamide cofactor (e.g., G-6-PD plus NADP), and in other cases, by the cosubstrate (e.g., isocitrate dehydrogenase plus isocitrate). Precisely the same protection could be obtained when compounds that react with sulfhydryl compounds (e.g., p-mercuricbenzoate) were used instead of PAN. Thus, the evidence indicated that PAN reacted with sulfhydryl groups.

Direct evidence of the reaction of PAN with sulfhydryl compounds has since been obtained (PAN at 115 ppm for 1-10 min).[306, 388, 391] In the reaction with glutathione, the major products are oxidized glutathione (disulfide) and S-acetylglutathione. Other sulfhydryl compounds (e.g., coenzyme A, lipoic acid, and cysteine) yield only oxidation products, with no evidence of S-acetylation. However, acetylation reactions have been observed with alcohols[403] and amines.[583] Sulfur compounds other than thiols can undergo oxidation by PAN: methionine is converted to methionine sulfoxide, and oxidized lipoic acid (disulfide) is converted to sulfoxide.

Papain is readily inactivated by PAN (at 115 ppm for 40 min), provided that it is in the sulfhydryl form.[391] The reaction of sulfhydryl groups of hemoglobin with PAN is very similar to the reaction with p-mercuricbenzoate: there is more reaction at a pH of 4.5 than at a pH of 7. However, there is one striking difference between PAN and classic compounds that react with sulfhydryl groups: egg albumen is resistant to reaction with PAN.[391] Thus, enzymes that have no free sulfhydryl groups should be quite resistant to PAN. This is the case with pancreatic ribonuclease: the native enzyme was not affected by a 300-fold molar excess of PAN.

There is some difficulty in correlating results of PAN exposure in vivo with the above types of in vitro studies. For example, UDP-glu-pyrophosphorylase is very susceptible to PAN in vitro, but in vivo there is a stimulation of enzymatic activity (at 112 ppm for 6 min).[186] It is also anomalous that, whereas phosphoglucomutase of oat seedlings is sensitive to PAN both in vivo and in vitro (at 50 ppm for 4 h),[186] it is generally considered that the active site is serine, rather than cysteine. Results of enzyme analysis after in vivo exposure to tissues to PAN are complicated, because direct effects on the enzyme may be overshadowed by effects on protein synthesis, and these effects in turn may be directly on the protein synthesizing machinery or on the regulatory mechanisms that depress or stimulate protein synthesis. A schematic representation of this dynamic system shows that PAN could affect the synthetic process (site 1), the enzyme itself (site 2), or the degradation process (site 3). If the site of attack were site 2, the synthetic process might compensate for degradation of the enzyme by producing more.



If the enzyme activity were measured as a function of time after exposure, there would be first a decrease and then recovery of activity. (Such a response has been observed for the effect of ozone on respiration.) Effects at site 3 would show first an increase in activity and then, if the system were regulated, a decline to normal, as the synthetic process slowed down. *Effects at site 1 would cause a decrease in activity commensurate* with the rate of enzyme degradation.

Experiments with chloroplasts showed an apparent inhibition of fatty acid synthesis by PAN (at 72 ppm for 10 min).[389] The result is difficult to interpret: the inhibition could be attributed to inactivation of one of the enzymes of the multistep system or to oxidation of the reductant (reduced NADP, or NADPH) required in the chain elongation process.

In addition to the protein and low-molecular-weight thiols that react with PAN, there are several other reactive biochemicals. Reduced nicotinamide derivatives are susceptible to oxidation by PAN (at 72 ppm for 1-5 min),[389] whereas the oxidized forms are resistant. The capability of PAN to oxidize these compounds rapidly dissipates in aqueous solution, with a half-life of 4-10 min, depending on pH. The oxidation products appear to be the biologically active forms of the nicotinamide derivatives. Purines and pyrimidines react with PAN (at 1,000 ppm for 30-120 min).[427] The order of sensitivity is thymine, guanine, uracil, cytosine, and adenine. Their reactions were studied at relatively low pH and at high PAN concentration and are probably not of biologic significance.

Various olefins and amines (undiluted) react with solutions of PAN in $CdCl_3$:[104, 583]

$$PAN + R_2C = CR_2 \longrightarrow R_2C\overset{O}{\diagup\diagdown}CR_2 + MeONO + MeNO_2 ;$$

$$PAN + RNH_2 \longrightarrow CH_3CONHR + O_2 + HNO_2 .$$

These oxidation and acetylation reactions have not yet been examined in a biologic context.

- Chemical basis of toxicity: The toxicity of the peroxyacylnitrates to plants can be the result of several different processes. Any chemical

explanation of the toxicity will have to explain the increasing toxicity of the series PAN, PPN, PBN, and PBzN. Three hypotheses have been tested:[390] the more toxic homologues are more readily taken up by the plant; all homologues are taken up equally well, but the plant is able to reverse the actions of the lower homologues; and all homologues are taken up equally well, but the higher homologues have longer half-lives. In testing the first hypothesis, it was found that PAN and PPN were taken up equallly well by plants; so that hypothesis is probably incorrect. The ability of the plant to hydrolyze thioesters of glutathione was tested; the second hypothesis predicted that the higher homologues would be hydrolyzed more slowly; but the opposite was found.[591] Thus, the second hypothesis, although not fully tested (in that the thioesters measured may not be the ones formed in the cell), does not look promising. The third hypothesis was tested by determining the half-lives of the oxidizing power of each compound in aqueous medium. PBN had the longest half-life, as predicted, but the half-lives of PAN and PPN were about the same.[390] It seems probable that half-life influences the toxicity of the peroxyacylnitrates, although definitive results have not yet been obtained.

Discussion. The acute responses of plants to ozone and PAN result from disruption of normal cellular mechanisms. The initial response causes disorganization, prehaps through an attack on sulfhydryl linkages or unsaturated lipid linkages. Cellular water and salts are lost, with cell plasmolysis resulting. Cell death normally will occur; however, depending on dose and environmental conditions, membrane permeability may be restored and cell recovery take place. The extent of recovery depends on the severity of the external stresses and probably on the ability of the cells to initiate repair

mechanisms. A sigmoid response has been observed in many plants exposed to ozone and probably reflects physical resistance to gase movement within the tissues. Tingey et al.[544] determined activity changes in selected enzymes from soybean leaves and found that the reactions were similar to those produced by other stresses, and not to the process of senescence.

Chronic injury results primarily from secondary reactions involving membrane injury. The oxidants could cause the formation of free radicals or other, more stable oxidants (such as hydrogen peroxide), which in turn could cause secondary reactions. These secondary reactions could stimulate the production of cellular ethylene, with tissue senescence resulting.[1, 89, 539] These secondary reactions may predispose plants to increased injury from later acute exposures by limiting their repair capability. This predisposition concept has been noted in several reports.

Reproductive Systems and Life Cycles

Ozone and PAN are both strong oxidizing agents. Some work has suggested that ozone is a radiomimetic gas. It is known to cause chromosomal breakage in both plants and animals at high concentrations. Although the potential of ozone's mutagenic effects on vegetation at ambient concentrations is not understood, Sparrow and Schairer[491] reported that ozone appeared to be a weak mutagen in causing an increase in the pink somatic mutation rates in petals of Tradescantia.

Feder[155] showed an effect of ozone on tobacco pollen germination and postulated effects on reproductive capacity of tobacco. This work was not substantiated by Sinclair,[488] but the exposure techniques used were questionable. Gentile et al.[177] showed that germination of pollen from

sensitive ascessions of Lycopersicon Pimpinellifolium were reduced by as much as 40% with techniques described by Feder.[155] However, reciprocal cross-pollinations between ozone-exposed and -unexposed populations led to normal fruit development and viable seeds. Harrison and Feder[193] found a migration of cell organelles from the outer layer of cytoplasm in pollen grain of sensitive petunia exposed to ozone. They used 0.50 ppm for 3 h and found an 80% reduction in germination. Mumford et al.[397] found that 0.03-0.06 ppm was the threshold for an effect on germination of corn pollen. They did extensive biochemical studies of the pollen and suggested that ozone induces autolysis of structural glycoproteins and stimulates amino acid synthesis. Cameron and associates[62,63] found major reductions in yield of some corn varieties after high-ambient-oxidant episodes occurred during tasseling. These reductions appeared to result from poor fertilization. Although preliminary, those studies point to the importance of understanding the direct effects of oxidant pollutants on reproductive processes within plant species.

A study was undertaken to determine the potential mutagenic effects of ozone on Arabidopsis thaliana.[57] This plant completes its life cycle in about 35 days and is an excellent test species for determining effects of ozone across life cycles. Plants were exposed to acute doses for 6 h/day, 3 days/week, through 4 weeks of the life cycle. Seeds were collected from control and exposed plants and planted over seven generations. Seed production and biomass were reduced within generations, but no factors studied were carried over into later generations. The results showed that, for this particular species and the concentrations used, no mutagenic effects were carried via seed to later generations. This was a preliminary study that also reported

problems with variability in plant response. The author suggested that similar studies be initiated to verify these results.

Biomass and Yield

Photochemical oxidants, including ozone and PAN, reduce plant biomass and yields in many plant species. Although yield and growth reductions are normally associated with visible injury, the oxidants can cause growth reductions with little or no injury. This relationship is not understood for the oxidant pollutants and probably varies with species and cultivars, as well as with conditions of growth and exposure, including dose. Todd and Arnold[552] compared injury with biomass and chlorophyll content for pinto bean exposed to a synthetic oxidant (ozone plus hexene). They found a logarithmic relationship between injury and the two physiologic measurements and suggested that injury was not a reliable index of growth effects. Their data were from acute exposures and suggest effects on growth at low injury values. A group of Canadian workers[4, 5] have used physiologic measures exclusively for acute exposures. Many investigators are using physiologic measures, instead of injury, because they are more objective. Although physiologic responses are often better measures of chronic effects, their variability is usually at least as great as that of a subjective injury measure, and they are sometimes not as sensitive in indicating acute effects. Runeckles and Resh[470] reported on a reflectance measure of chronic effects that was much less variable than chlorophyll extraction. They did not compare this with a visual injury estimate. This technique should be studied further.

Considerable information is available on growth and yield effects. However, because it comes from scattered sources and much of it is

subjective, no one has developed the data for predictive use (relating dose or injury to yield reductions). This section is divided into a discussion of effects under ambient conditions and a discussion of effects with controlled additions of oxidants, primarily ozone. The ambient oxidant section includes observational information and chamber studies comparing plant response in filtered versus unfiltered ambient air.

Ambient oxidant studies. Since the first observations of oxidant injury,[369] there have been continual reports of effects of oxidants on vegetation. This section does not attempt to cover all those reports, but does give several descriptive examples. The closing sections of this chapter (on plants as monitors and on economics) have additional examples. Engle et al.[144] first identified ozone as the cause of onion tip burn. They associated the development of this condition with severe weather fronts and suggested that ozone, produced in thunderstorms, may be the culprit. They identified both resistant and sensitive cultivars where resistance was genetically controlled. Weaver and Jackson[580] first described bronzing in white bean and associated this with oxidant (ozone) episodes. They found that ozone produced similar symptoms and that 0.16-ppm ozone for 0.5 h was the threshold for effects in their studies. This disease is known to cause reductions in bean yields.[184] Haas did a growth analysis in field plot studies. He selected 5 plots based on severity of bronzing symptoms and showed a relationship between severity of bronzing and growth. He found that the rate of growth influenced symptom severity and that the stage of growth determined the dose at which bronzing occurred. He showed that physical conditions within a field could cause a variable response.[42] Brasher et al. reported 10 - 95% injury to 3 cultivars and 16 seedling lines of potato

after 3 days of ambient oxidant that reached a maximum of 0.15 ppm. No yield data were reported for this study. Rich et al.[459] reported crop damage from oxidants to many plants in Connecticut when oxidant concentration was above 0.05 ppm. There were 83 of these high-oxidant days in 355 days monitored over 4 growing seasons. Reinert et al.[451] found similar effects around Cincinnati, Ohio, when oxidant concentrations were high. In this study, a group of plants was set out to monitor effects. Generally, these studies have not included quantitative yield data. Oshima et al.[422] reported a severe episode in California where injury was greater than expected on the basis of total-oxidant measurements. The presence of PPN was suspected. This is indicative of the episodic potential in high-pollution areas throughout the country. Several studies have reported on the severe effects of oxidants on ponderosa pine in the San Bernardino Mountains,[77, 375] and quantitative work (including growth-ring analyses) is reported elsewhere in this document.[32, 571] Several subjective observations have come from Europe and Australia,[588] but little quantitative work has come from outside the United States and Canada.

Several excellent studies have reported yield data obtained by use of filtered and nonfiltered greenhouse or field chambers. Selected summary data are shown in Table 11-2. The first field-chamber work was reported on citrus by Thompson and Taylor.[521] Their report summarized several years of detailed work on lemon and orange and considered many combinations of pollutants. Reductions in fruit size, number, and total yields were reported in these studies and are the basis for the projected 50% reductions in citrus yields from the Los Angeles basin.[520] Thompson and Kats showed that some of these effects on citrus were probably related to concentrations

Table 11-2

Effects of Ambient Oxidants (Ozone) on Growth, Yield, and Foliar Injury in Selected Plants[a]

Plant Species	Oxidant Concentration, ppm	Exposure Time	Plant Response, % reduction from control	Reference
Lemon	> 0.10	Over growing season	32, yield 52, yield (leaf drop and other effects)	521
Orange	> 0.10	148 h/month ave. in March-Oct.; 254 h/month ave. in July-Sept.	54, yield (other reductions found)	521
Grape, cultivar Zinfandel	≥ 0.25	Often over May-Sept. growing season	12, yield (first year) 61, yield (second year) (increased sugar content) 47, yield (third year)	522 519 518
Corn, sweet	0.20-0.35	Hourly maximum for 3-4 days before injury	67, injury (10 cultivars, 5 unmarketable) 18, injury (13 cultivars) 1, injury (11 cultivars)	63
Bean, white	> 0.08	9 h	(Bronze color, necrotic stipple, premature abscission)	580
Tobacco, cultivar White Gold	0.20 ppm-h[b]	--	(Threshold for sensitive plants)	324
Tobacco, cultivar Bel W 3[c]	0.02-0.03	6-8 h	(Minimal--threshold--injury)	212
Tobacco, cultivar Bel W 3	> 0.05	Often over growing season	22 (fresh wt), top 27 (fresh wt), root	204

Table 11-2 (Cont.)

Effects of Ambient Oxidants (Ozone) on Growth, Yield, and Foliar Injury in Selected Plants[a]

Plant Species	Oxidant Concentration, ppm	Exposure Time	Plant Response, % reduction from control	Reference
Soybean, 4 cultivars	≥ 0.05	465 h during growing season	28 (16 - 40), yield	253
Cotton, cultivar Acula	Ambient	Over growing season	7-20, lint + seed (3 locations, 1972) 5-29, lint + seed (3 locations, 1973)	53
Potato, 4 cultivars[c]	≥ 0.05	326-533 h (2 years)	34-50, yield (2 years for 2 cultivars) 20-26, yield (1 year for 2 cultivars)	221
Potato, cultivar Haig	0.15	3 consecutive days	95, injury (leaf area covered)	42

[a]Many field observations of injury to vegetation have been reported in the literature since Middleton et al.[369] first reported effects in California. Most are reported without quantitative data on pollutant dose or plant effects and give descriptions of plant injury.[42,144,184,422,451,459,580] Good summaries are given in several reviews.[211,223,235]

[b]Dose = concentration x time.

[c]Greenhouse studies.

of PAN in the ambient air. Thompson and co-workers [518, 519, 522] used the same chambers to determine effects of oxidants on grape over 2 years. They found nominal effects in the first year and 50-60% reductions in the next 2 years. The differences were attributed to the effects of oxidants on floral initiation in the year before the first study year. This concept could hold for any plants when floral structures are initiated in the prior season. They also reported a 41-62% reduction in leaf chlorophyll and a 13-17% reduction in sugars. A wax emulsion spray gave a 20% increase in yield over unsprayed plants; this suggests that selected protectants may be useful in protecting grapes grown in areas of high oxidant concentration. These chambers were also used in the San Joaquin Valley in California to determine effects of oxidants on cotton.[53] Yield reductions of 5-29% in the lint and seed were reported at three locations over 2 years, but no effects on lint quality or seed grade were found. Heggestad[221] reported reduced yields in four potato cultivars grown in filtered versus nonfiltered greenhouses. The results cover a 3-year study and show up to a 50% loss in yield from sensitive cultivars. He also presented an excellent description of oxidant and ozone symptoms on potato. Heagle et al.[204] reported preliminary results on yield reduction in tobacco grown as part of a test of an open-top field-chamber design. They found significant reductions in both top and root fresh weight that compared closely with injury ratings (Table 11-3). Howell and Koch[253] reported yield reductions of 16-40% in four soybean cultivars with these open-top chambers. They found no changes in the protein or oil of seed.

Table 11-3

Injury versus Growth Reduction in Selected Plants Exposed to Ozone

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Growth Response, % reduction from control	Foliar Injury Response, % increase over control	Reference
Bean, cultivar Pinto	0.30	0.5/day, 14 days	11, leaf dry wt	9	319
	0.30	1.0/day, 14 days	40, leaf dry wt	46	
	0.30	2/day, 14 days	70, leaf dry wt	69	
	0.30	3/day, 14 days	76, leaf dry wt	78	
Corn, sweet	0.05	6/day, 64 days	12, ave. 4 yield responses	14	206
	0.10	6/day, 64 days	35, ave. 4 yield responses	25	
Soybean, cultivar Dare	0.05	6/day, 133 days	22, plant fresh wt	19	205
	0.10	6/day, 133 days	65, plant fresh wt	37	
	0.1 (+ SO_2 at 0.1)	6/day, 133 days	75, plant fresh wt	46	
Tobacco, cultivar Bel W_3	> 0.05 [a]	(Often over growing season)	22, top fresh wt	17	204
Soybean (2 cultivars)	0.05	8/day, 5 days/wk, 3 wk	2, top fresh wt	8	548
	0.10	8/day, 5 days/wk, 3 wk	21, top fresh wt	19	

[a] A study in field chambers with ambient photochemical oxidant (ozone) concentrations.

Controlled chamber studies. Many workers have used controlled additions of ozone in acute or chronic studies to determine effects on a variety of growth measures. These studies suggest results attributable to ambient oxidants in field plantings. However, in the field, there are probably combinations of acute, chronic, and predisposition effects of oxidants on the susceptible species and cultivars. Most of these studies are from greenhouse and controlled-chamber exposures, but several have used chambers placed over field plantings. Several studies have shown correlations between injury and yield or biomass reductions (Table 11-3). These suggest that injury may be a good criterion of yield reductions. However, it is known that yield reductions occur with little or no visible injury and that injury may cause no measurable yield reductions. These studies have been divided according to whether they used short- or long-term exposures.

- Growth effects of short ozone exposures: Ordin and Propst[413] used high concentrations of either ozone or PAN and reported a 50% inhibition in indoleacetic acid-stimulated growth in Avena coleoptile. This suggested that the oxidant may have a direct effect on hormonal production. Adedipe and associates reported reduced biomass and floral production in four bedding plants[5] and in 2 radish cultivars[4] from acute ozone exposures (Table 11-4). They reported no effects on marigold, celosia, impatiens, and salvia, even at the high concentration of 0.80 ppm for a 2-h exposure. The radish study included effects of exposure temperatures on growth. One cultivar reacted the same across temperatures, but the response of the second was conditioned by temperature. Tingey et al.[540] exposed radish at 7, 14, or 21 days and all combinations of these ages to ozone at 0.40 ppm for 1.5 h. They found the greatest effect on root growth at 14 days for the single

Table 11-4

Effects of Acute Ozone Exposure on Growth and Yield of Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control[a]	Reference
Begonia, cultivar White Tausendschon	0.10	2	5, ave. of 3 growth responses: shoot wt, flower wt, flower no.	5
	0.20	2	10, ave. of same responses	
	0.40	2	19, ave. of same responses	
	0.80	2	38, ave. of same responses	
Petunia, cultivar Capri	0.10	2	9, ave. of same responses	5
	0.20	2	11, ave. of same responses	
	0.40	2	21, ave. of same responses	
	0.80	2	31, ave. of same responses	
Coleus, cultivar Scarlet Rainbow	0.10	2	2, ave. of same responses	5
	0.20	2	17, ave. of same responses	
	0.40	2	24, ave. of same responses	
	0.80	2	39, ave. of same responses	
Snapdragon, cultivar Floral Carpet, mixture	0.10	2	0, ave. of same responses	5
	0.20	2	6, ave. of same responses	
	0.40	2	8, ave. of same responses	
	0.80	2	16, ave. of same responses	
Radish, cultivar Cavalier, Cherry Belle	0.25	3	36, top dry wt (Cavalier) 38, root dry wt (Cherry Belle)	4
Radish	0.40	1.5(1)[b]	37, root dry wt	540
		1.5(2)[b]	63, root dry wt	
		1.5(3)[b]	75, root dry wt	
Cucumber, cultivar Ohio Mosaic	1.00	1	19, top dry wt (1% injury)	416
	1.00	4	37, top dry wt (18% injury)	

Table 11-4 (Cont.)

Effects of Acute Ozone Exposure on Growth and Yield of Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control[a]	Reference
Potato, cultivar Norland	1.00	4	0, tuber dry wt (no injury)	416
	1.00	4 (3)[b]	30, tuber dry wt (injury severe)	
Tomato, cultivar Fireball	0.50	1	15, plant dry wt (grown in moist soil)	280
	1.00	1	20, plant dry wt (grown in moist soil)	
Tomato, cultivar Fireball	0.50	1	15, increase in plant dry wt (grown in dry soil)	280
	1.00	1	25, increase in plant dry wt (grown in dry soil)	
Onion, cultivar Spartan Era	0.20	24	0, effect	416
	1.00	1	19, plant dry wt (no injury)	
	1.00	4	49, plant dry wt	
Tobacco, cultivar Bel W_3	0.30	2	48, chlorophyll content	6

[a] Unless otherwise noted.

[b] Number of exposures in parentheses.

exposure and at 7 and 14 days for the double exposure. The greatest effect was found in the root growth from the triple exposure (7, 14, and 21 days). The root reductions for the multiple exposures were equal to the additive reductions for single exposures. It was concluded that reductions were a result of photosynthate going preferentially to top growth. Reduced root growth rate was still found in the second week after exposure.

The studies of acute effects resulted in interesting growth phenomena that require further study. Tingey et al.[542] related foliar sensitivity in the first trifoliate leaf of soybean to leaf growth, stomatal resistance, and several metabolites. Maximal sensitivity occurred during the end of the period of rapid leaf expansion, when stomatal resistance was low. However, reduced sensitivity was shown in older leaves when stomatal resistance was still low. They found no relationship between sensitivity and concentrations of protein, amino acids, or various carbohydrates. This suggested that metabolic pools were not directly related to foliar response. Tingey and Blum[536] reported a consistent reduction in root growth that was related to reduced nodule number in soybean. The ozone did not affect nodular activity or size. Evans[147] exposed pinto bean to acute ozone doses and found a reduction in leaf expansion within 12 h (10%) and a 33% maximal reduction on the day after a 0.20-ppm exposure. Growth rate continued to slow 3 days after exposure. He used the terminal leaflet of the first trifoliate for these studies. A stimulation of stem elongation in tomato within 3 days after a 2-h exposure to 0.30-ppm ozone[402] and a predisposition to increased sensitivity from acute ozone exposure after exposure to low ozone concentrations[467] have also been reported. These are only some of the effects seen. Similar results are reported elsewhere in this chapter.

• Growth effects of long-term ozone exposures: Taylor et al.[512] first reported a 52% reduction in the fresh weight of avocado seedlings exposed to a synthetic "smog" (ozone plus hexene) for 280 h. Tingey et al.[548] found reduced growth in 2 soybean cultivars (Hood and Dare) after intermettent exposure to ozone at 0.10 ppm over 3 weeks. They found that root and top growth were equally affected, but that roots were most affected when soybean was exposed to a mixture of ozone and sulfur dioxide. They found similar results[545] with radish. Little injury was reported in either paper. Frey[171] reported a 55% reduction in seed yield of soybean with a 5% decrease in seed lipids and a 21% increase in free amino acids. This is one of the few reports on quality changes in seed as a result of ozone exposure. A 30% reduction in wheat yield occurred when wheat was exposed at anthesis to ozone at 0.2 ppm, 4h/day for 7 days.[481] Oshima[423] reported a reduction in tomato yield at 0.35 ppm over a long exposure period, whereas injury effects were significant at both 0.20 and 0.35 ppm. He suggested an injury-tolerance threshold for tomato below which one would find no yield reduction. He also reported a decrease in kernel weight of sweet corn exposed to ozone at 0.20 or 0.35 ppm.[418] The reduced weight was associated with a shrivel ear condition (kernels) that might relate to the effects of ozone on pollen development.

Craker[91] reported a reduction in petunia flower weight after a 53-day exposure to ozone at 0.05-0.07 ppm,[93] but an increase in petunia flower weight was found in a 7-day exposure to 3 different concentrations. Carnations continuously exposed to 0.07-0.08 ppm produced a single deformed flower, but the controls had 24 normal flowers.[157] Poinsettia bract area was decreased by 39% after a 50-day exposure (6 h/day) to ozone at 0.10-0.12 ppm.

Bennett et al.[22a] make a case for stimulation of growth at low concentrations of ozone. They exposed bean (cultivar Pure Gold Wax), barley (cultivar Brock), and smartweed to ozone at 0.03 ppm over some growth stage and found instances of significant growth increases. The concept needs further study in light of the current concern over "normal" background ozone concentrations.

Heagle and associates[206] found a reduction in yield of sweet corn and soybean[205] after exposure to ozone at 0.10 ppm for 6 h/day over much of the growing season. These exposures were carried out in field chambers set over soybean plots in the field. They suggested that a threshold for measurable effects on these crops would lie between ozone (oxidant) concentrations of 0.05 and 0.10 ppm for 6 h/day. These values are realistic in terms of growing-season averages in the eastern United States. More of these studies could help to clarify dose-response relationships for economically important crops.

Table 11-5 summarizes these long-term, chronic studies.

Harwood and Treshow[194] exposed 15 species, representative of the aspen plant community, to ozone at 0, 0.05, 0.15, and 0.30 ppm and ambient air during the growing season and reported effects in all species at the highest pollution concentration (Table 11-6). There was considerable plant variability, and only 6 species reproduced. However, vigor was reduced and most species were sensitive. Price and Treshow[441] found major biomass reductions in 6 grass and 2 tree species exposed for 4 h/day to ozone at 0.15-0.33 ppm over a growing season. They also found a reduction in or loss of some reproductive components. These effects could result in subtle shifts in community composition after several years of ozone exposure.

Table 11-5

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Lemna, duckweed	0.10	5/day, 14 days	100, flowering; 36, flowering (1 wk after exposure completed) 50, frond doubling rate	158
Carnation	0.05-0.09	24/day, 90 days	50, flowering (reduced vegetative growth)	156
Geranium	0.07-0.10	9.5/day, 90 days	50, flowering (shorter flower lasting time, reduced vegetative growth)	156
Petunia	0.05-0.07	24/day, 53 days	30, flower fresh wt	91
Poinsettia	0.10-0.12	6/day, 5 days/wk, 10 wk	39, bract size	93
Radish	0.05	8/day, 5 days/wk	54, root fresh wt 20, leaf fresh wt	545
	0.05	8/day, 5 days/wk (mixture of O_3 and SO_2 for same periods)	63, root fresh wt 22, leaf fresh wt	

Table 11-5 (Cont.)

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Beet, garden	0.20	3/day, 38 days	50, top dry wt 40, storage root dry wt 67, fibrous root dry wt	412
Bean, cultivar Pinto	0.13	8/day, 28 days	79, top fresh wt 73, root fresh wt 70, height	345
Bean, cultivar Pinto	0.05	24/day, 3-5 days	50, leaf senescence (chlorosis)	141,143
	0.05	24/day, 5 days	(fivefold increase in lateral bud elongation)	
Bean, cultivar Pinto	0.15	2/day, 63 days	33, plant dry wt; 46, pod fresh wt	244
	0.25	2/day, 63 days	95, plant dry wt; 99, pod fresh wt	
	0.35	2/day, 63 days	97, plant dry wt; 100, pod fresh wt	
Bean, cultivar Pinto	0.15	2/day, 14 days	8, leaf dry wt	319
	0.15	3/day, 14 days	8, leaf dry wt	
	0.15	4/day, 14 days	23, leaf dry wt	
	0.15	6/day, 14 days	49, leaf dry wt	
	0.225	2/day, 14 days	44, leaf dry wt	

(Data available on whole plants, roots, leaves, injury, and 3 levels of soil moisture stress)

Table 11-5 (Cont.)

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Bean, cultivar Pinto	0.225	4/day, 14 days	68, leaf dry wt (Data available on whole plants, roots, leaves, injury, and 3 levels of soil moisture stress)	319
	0.30	1/day, 14 days	40, leaf dry wt	
	0.30	3/day, 14 days	76, leaf dry wt	
Tomato, pole var	0.20	2.5/day, 3 days/wk, 14 wk	1, yield; 32, top dry wt; 11, root dry wt	423
	0.35	2.5/day, 3 days/wk, 14 wk	45, yield; 72, top dry wt; 59, root dry wt	
Corn, sweet, cultivar Golden Jubilee	0.20	3/day, 3 days/wk, till harvest	13, kernel dry wt; 20, top dry wt; 24, root dry wt	418
	0.35	3/day, 3 days/wk, till harvest	20, kernel dry wt; 48, top dry wt; 54, root dry wt	
Wheat, cultivar Arthur 71	0.20	4/day, 7 days (anthesis)	30, yield	481
Soybean	0.05	8/day, 5 days/wk, 3 wk	2, top fresh wt	548
	0.10		24, root fresh wt 21, top fresh wt	

Table 11-5 (Cont.)

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Soybean	0.05	(mixture of O_3 and SO_2 for same periods)	24, root fresh wt 12, top fresh wt 30, increase in shoot: root ratio [a] (greatest effect on roots)	548
Alfalfa	0.10	2/day, 21 days	16, top dry wt	487
	0.15	2/day, 21 days	26, top dry wt	
	0.20	2/day, 21 days	39, top dry wt	
Grass, brome	0.15-0.33 (varied)	4/day, 5 days/wk, growing season	83, biomass	441
Pine, eastern white	0.10	4/day, 5 days/wk, 4 wk (mixture of O_3 and SO_2 for same periods)	3, needle mottle (over 2-3 days of exposure) 16, needle mottle	125
Pine, ponderosa	0.15	9/day, 10 days	4, photosynthesis	379
	0.15	9/day, 20 days	25, photosynthesis	

Table 11-5 (Cont.)

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Pine, ponderosa	0.15	9/day, 30 days	25, photosynthesis	379
	0.15	9/day, 60 days	34, photosynthesis	
	0.30	9/day, 10 days	12, photosynthesis	
	0.30	9/day, 20 days	50, photosynthesis	
	0.30	9/day, 30 days	72, photosynthesis	
	0.45	9/day, 30 days	85, photosynthesis	
Poplar, yellow	0.30	8/day, 5 days/wk, 13 wk	82, leaf drop; 0, height	264
Maple, silver	0.30	8/day, 5 days/wk, 13 wk	50, leaf drop; 78, height	264
Ash, white	0.30	8/day, 5 days/wk, 13 wk	66, leaf drop; 0, height	264
Sycamore	0.30	8/day, 5 days/wk, 13 wk	0, leaf drop; 22, height	264

Table 11-5 (Cont.)

Effects of Long-Term Controlled Ozone Exposures on Growth, Yield, and Foliar Injury to Selected Plants

Plant Species	Ozone Concentration, ppm	Exposure Time, h	Plant Response, % reduction from control	Reference
Maple, sugar	0.30	8/day, 5 days/wk, 13 wk	28, leaf drop; 64, height	264
Corn, sweet, cultivar Golden Midget[b]	0.05	6/day, 64 days	9, kernel dry wt; 14, injury (12, ave. 4 yield responses)	206
	0.10	6/day, 64 days	45, 25, 35 for same responses	
Soybean, cultivar Dare[b]	0.05	6/day, 133 days	3, seed yield; 22, plant fresh wt; 19, injury	205
	0.10	6/day, 133 days	55, 65, 37 for same responses	
	0.10	(mixture of O_3 and SO_2 for same periods)	63, 75, 46 for same responses	
Poplar, hybrid	0.15	8/day, 5 days/wk, 6 wk	50, shoot dry wt; 56, leaf dry wt; 47, root dry wt	265

[a] The only increase.

[b] Studies conducted under field conditions, except that plants were enclosed to ensure controlled pollutant doses.

Table 11-6

Effects of Ozone on Selected Understory Species from an Aspen Community[a]

Plant Species	Plant Response at Different Ozone Concentrations[b]								
	Foliar Injury, %			Plant Wt, % of control			Seed Wt, % of control		
	0.05 ppm	0.15 ppm	0.30 ppm	0.05 ppm	0.15 ppm	0.30 ppm	0.05 ppm	0.15 ppm	0.30 ppm
Chenopodium album L.	0	35	40	103	71*	83	87	99	87
C. fremontii L.	10	35	90	71	98	32	102	96	94
Descurainia sp.	0	15	55	179*	77	56*	-	-	-
Geranium fremontii, L. Torr. ex A. Gray	7	50	90	93	94	53*	-	-	-
Lepidium virginicum L.	50	100	100	121	72	31*	94	69	47*
Madia glomerata	40	100	100	112	38*	13*	79	74	18
Polygonum aviculare L.	30	95	95	271	186*	29*	127	43	50
P. douglasii Greene	5	35	95	87	29*	5*	93	84	3*

[a]Exposures were 3 h/day, 5 days/wk, through growing season. Data from Harward and Treshow.[194]

[b]Asterisk indicates significant effect.

The results of these experiments suggest that overall economic effects on agricultural production could be extensive, depending on the sensitivity of cultivars used in production, and should be considered in the attempt to arrive at valid economic-damage functions for vegetation.

Factors Affecting Plant Response

The sensitivity of plants to ozone and PAN is conditioned by many interrelated factors. Heck[210] reviewed these factors and found that our understanding of the importance of any given factor on plants was fragmentary. Since then, a new body of research has become available that permits a more concise discussion of many factors that affect the response of plants. Although the response of a given plant is not predetermined by the response of related plants, sufficient information to predict relative responses under given sets of conditions is available. Information to predict interactions between variables and to predict whether species respond the same way to environmental factors under different pollutant stresses is not available. Current research demonstrates our inadequacies in understanding interacting variables.

Before we can predict the response of a plant variety to a specific pollutant or group of pollutants, we must understand the following factors: genetic variability (both between and within species), climatic and edaphic factors, interactions with other pollutants, interactions with biotic pathogens and insects, and the growth and physiologic age of susceptible plant tissue. The overall conceptualization of relationships between pollutant exposure and ultimate effects is shown simplistically (Figure 11-1) in an adaptation from van Haut and Stratmann.[572]

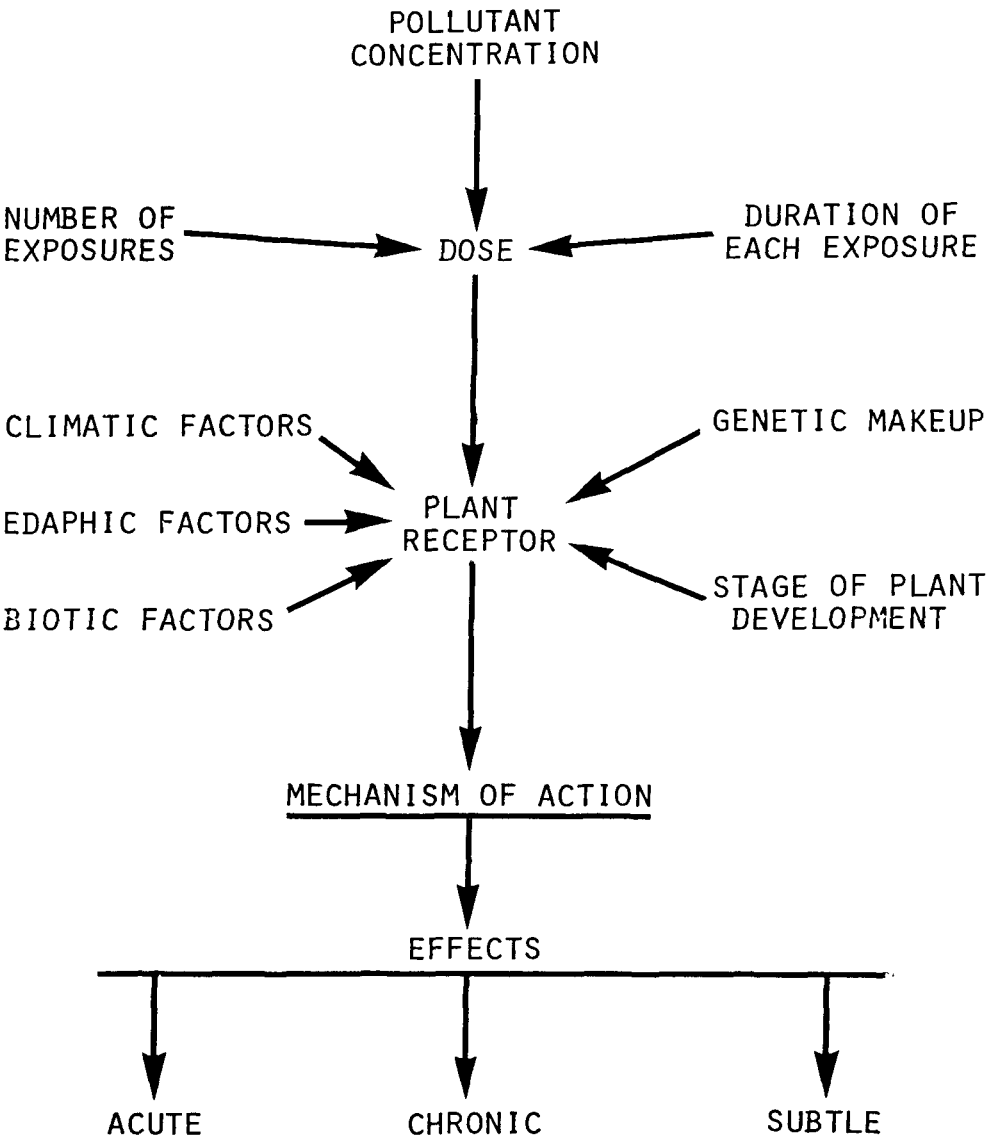


Figure 11-1. Conceptual model of factors involved in air pollution effects on vegetation. Modified from van Haut and Stratmann.[572]

Genetic Factors. Knowledge concerning the influence of genetic variability on plant response to ozone has been obtained from both field observations and chamber experimentation since the mid-1960's. Similar information on PAN is scarce, but several published reports are available. Resistance to ozone and PAN varies between species of a given genus and between cultivars within a given species. Variations in response are functions of genetic variability and environmental stress as they affect morphologic, physiologic, and biochemical characteristics. In native populations and in breeding experiments, both ozone and PAN may act as selective pressure stresses.

Variations in species response to ozone and PAN are well documented.[235,236,269,301,401,506,510,593a] A list of species susceptibilities to ozone is found in Table 11-24, which was generated from results of controlled exposures and the use of the sensitivity table, Table 11-23. Heck[210] summarized variations in cultivar responses of several species. Reinert[445] developed a compendium of research papers covering horticultural cultivar responses to ozone, PAN, and other pollutants that is complete, but gives no summary of results. A number of papers have been published since Brennan et al.[49] first published their results on the separation of oat and potato cultivars exposed to ozone and stressed the importance of cultivar differences within species. Additional ozone studies are available for bean,[109] begonia,[5] morning glory,[400] chrysanthemum,[48,284] poinsettia,[340] spinach,[338] lettuce,[449] radish,[449] turfgrass,[44,52] forage legume,[257] alfalfa,[256] safflower,[254,382,546] soybean,[477] small grain (oat, rye, wheat, barley),[47] and English holly. Petunia was studied with auto exhaust and PAN,[162] and both petunia and chrysanthemum

have been studied with PAN.[128,593] Screens using ambient oxidant conditions, sometimes without pollutant concentration, are fairly common: bean,[247] potato,[42,247,310] tobacco,[226,247,355,362] petunia,[310] poinsettia,[310] sweet corn,[63] and grape.[273] Most of these studies used acute exposures, which may or may not relate to results from chronic exposures. Some reports of ambient oxidant exposures covered part or all of a growing season. These screens used injury as the response measure. We do not know whether foliar injury ranking relates to economic yield loss ranking.

Cultivar variations in three species have been intensively studied in relation to their sensitivity to ozone: petunia,[5,67,162] tomato,[75,76,177,448] and tobacco.[115,181,226,239,358,361,362] These studies have resulted in recommendations that some cultivars not be used in areas of high oxidant potential. The susceptible Bel W$_3$ tobacco is widely used as a biologic indicator of oxidant.

A summary of cultivar responses to oxidants, ozone, and PAN is given in Table 11-7. Summary data were also included in the development of the dose-response equations and Table 11-24 on plant sensitivities.

Hanson[188] presented a list of 160 woody species from observations at the Los Angeles State and County Arboretum that were sensitive or tolerant to oxidants. Several recent investigations have considered susceptibility of tree species to ozone. Jensen[264] studied nine hardwood species, Berry[25] looked at three pine species, Davis and Wood[107,112] reported on 18 coniferous species, and Miller[373] reported on 15 western conifers. In general, selection within natural tree species has not been studied.

Table 11-7

Response of Cultivars within Species to Ozone and PAN

Plant Species	No. Cultivars	Pollutant and Concentration, ppm, h	Plant Response, % reduction from control (min.-mean-max.)	Sensitive, Resistant Cultivars	Reference
Oat	9	O_3, 0.22, 4	0 - 38 - 69 (injury)	C.I. 7540, C.I. 7271	49
Potato	8	O_3, 0.23, 4	0 - 15 - 50 (injury)	Chippewa, Avon	49
Bean	10	O_3, 0.25, 4	1 - 8 - 33 (injury)	Sanilac, Tendercrop	109
Bean	10	PAN, 0.12, 2	9 - 37 - 64 (injury)	Sanilac, Tempo	495
Potato	6	Oxidant, season[a]	Tolerant to susceptible (injury)	Norchip, Katohdin	247
Potato	24	Oxidant, 15, 3-day peak	10 - 54 - 98 (injury)	Haig, Superior	42
Petunia	8	Oxidant, season[a]	0 - 15 - 21 (top fresh wt)	Fiesta, Purple Plum	310
Begonia	3	O_3, 0.80, 2	7 - 39 - 50 (top dry wt)	White Tausendschon, Scarletta	5
Petunia	3	O_3, 0.80, 2	9 - 19 - 40 (top dry wt)	Capri, Canadian ALL Double Mix	5
Petunia	14	O_3, 1.00, 1	1 - 23 - 38 (injury)	Festival, Lilac Time	162
Petunia	6	PAN, 0.25, 1	4 - 15 - 24 (injury)	Blue Danube, Parti Pink	162
Petunia	15	Oxidant, 0.15, 1.5	2 - 12 - 45 (injury)	White Cascade, Parti Pink	162
Petunia	28	PAN, 0.15, 1	0 - 9 - 44 (injury)	Snow drift, Coral Magic	128
Chrysanthemum	45	O_3, 0.60, 3	0 - 23 - 70 (injury)	Tranquility, Ann Ladygo	284
Chrysanthemum	8	PAN, 0.60, 4	0 - 0 - 5 (injury)	None, all eight	593
Chrysanthemum	16	O_3, 0.40, 4	10 - 25 - 65 (injury)	Golden Arrow, Indian Summer	48
Poinsettia	5	O_3, 0.35, 4	6 - 35 - 63 (injury)	Eckespoint C-1, White Annette Hegg	340
Spinach	6	O_3, 0.15, 4	5 - 25 - 50 (injury)	Virginia Blight Res. Savoy, Bounty	338
Lettuce	8	O_3, 0.70, 1.5	10 - 24 - 46 (injury)	Dark Green Boston, Great Lakes	449
Radish	9	O_3, 0.35, 1.5	17 - 25 - 35 (injury)	Cherry Bell, Icicle	449

Table 11-7 (Cont.)

Response of Cultivars within Species to Ozone and PAN

Plant Species	No. Cultivars	Pollutant and Concentration, ppm, h	Plant Response, % reduction from control (min.-mean-max.)	Sensitive, Resistant Cultivars	Reference
Tomato	12	O_3, 0.40, 1.5	10 - 35 - 89 (injury)	Roma VF, VF 145 B	448
Tomato	24	O_3, 0.25, 1.5	5 - 50 - 75 (injury)	Roma VF, Heinz 1439	75
Tomato	63	O_3, variable	Injury	L. esc. (247089), L. pimp. (79532)	177
Tomato	295	O_3, 0.25, 1.5	0 - 40 - 75 (injury)	Various	76
Turfgrass	11 (7 spp.)	O_3, 0.30, 6	0 - 60 - 90 (injury)	Meyer Zoysia, Penncross	44
Forage legumes	10 (6 spp.)	O_3, 0.25, 4	0 - 10 - 50 (injury)	Crownvetch, Kenland Red Clover	52
Alfalfa	14	O_3, 0.20, 4	12 - 29 - 52 (injury)	Mesa - Sirsa, MSB - $CW5A_n2$	257
Safflower	12	O_3, 0.25, 2	32 - 65 - 73 (injury)	Nebraska - 4051, Frio	256
Soybean	21	O_3, 0.50, 2	45 - 64 - 79 (injury)	Wye, PI 157474	382
Soybean	14	O_3, 0.70, 1.5	17 - 40 - 57 (injury)	Hawkeye, Hood	546
Soybean	5	O_3, 0.50, 2	16 - 57 - 89 (injury)	Kent, Cutler	254
Sweet corn	34	Oxidant, >0.30, 3-day peaks	5 - 44 - 98 (injury)	No names	63
Tobacco, flue-cured	12	O_3, 0.20, 3	21 - 45 - 59 (injury)	Speight G-5, Coker 187 - Hicks	358
Tobacco, flue-cured	15	O_3, 0.36, 2.5	38 - 68 - 87 (injury)	Speight G-36, Coker 187 - Hicks	358
Tobacco, variable	16	Oxidant (Beltsville)[a]	1 - 16 - 65 (injury)	Pa 60-19, Bel W_3	355
Tobacco, wrapper	5	O_3, 0.06, ?	10 - 20 - 30 (injury)	Conn. 49, Dixie Shade	115
Tobacco, spp. and and cultivars	5	O_3, 0.30, 1	6 - 24 - 52 (injury)	N. glutinosa, Samsun (NN)	181
Tobacco	6	O_3, 0.25, 6 (3 days)	4 - 9 - 42 (injury)	Bel - C, Bel - B	226
Tobacco	5	Oxidant (Beltsville)[a]	4 - 51 - 73 (injury)	Bel - C, Madole	226
Tobacco	4	O_3, 0.20, 1.5	17 - 32 - 37 (injury)	Havana 142, Havana 501	239

Table 11-7 (Cont.)

Response of Cultivars within Species to Ozone and Pan

Plant Species	No. Cultivars	Pollutant and Concentration, ppm, h	Plant Response, % reduction from control (min.-mean-max.)	Sensitive, Resistant Cultivars	Reference
Tobacco	6	O_3, 0.15, 3	27 - 35 - 38 (injury)	Catterton, Wilson	362
Grape	134	Oxidant (N.Y. and Ontario)[a]	0 to severe	Ives, Delaware	273
Holly	2	O_3, 0.50, 7	No effects	English holly (resistant)	47

[a] Concentration present at location, not given.

Two studies have explored the mechanism of genetic resistance to ozone. Engle[141] and Engle and Gabelman[142] found that ozone sensitivity in onion is probably controlled by a single gene pair, with dominance of the resistant gene. In resistant plants, the membrane of the guard cells was more sensitive to ozone and lost its differential permeability, thus causing stomatal closure. This did not occur in the guard cells of the sensitive cultivar, and thus the stomata remained open. Taylor[500] crossed the sensitive Bel W 3 tobacco with a resistant line and found that the F_1 was of intermediate sensitivity. The F_2 segregated into 40% resistant, 10% sensitive, and 50% intermediate. He postulated that sensitivity was controlled by at least two genes. Resistance mechanisms need to be studied in other species. Dean[116] reported a 50% increase in stomatal density of two sensitive tobacco cultivars over two resistant cultivars. Stomatal size was not a factor in this study.

Differential susceptibility of individual clones of eastern white pine to ozone and sulfur dioxide was shown by Berry and Heggestad[28] and Costonis.[82] When Dochinger et al.[126] determined that chlorotic dwarf could be caused by an interaction of ozone and sulfur dioxide, they used a chlorotic dwarf-susceptible clone to eliminate the genotype variable. Houston[249] tested the response of tolerant and susceptible clones of eastern white pine (on the basis of symptom expression under ambient conditions) to ozone or sulfur dioxide. Injury caused by sulfur dioxide or sulfur dioxide plus ozone correlated well with the earlier field responses, but ozone did not produce a consistent response. They also found that a 6-h exposure to a mixture of sulfur dioxide and ozone caused a difference in needle elongation between clones within tolerant and sensitive groups. This suggests that tollerance may function over a wise range of responses.

It is reasonable to suggest that a spectrum of genotypes represents various susceptibilities to oxidants, singly or in combination. Although environmental factors are important in conditioning pollutant susceptibility, the control of environment over injury to sensitive genotypes may be less pronounced, if specific biochemical or physiologic mechanisms that have weak interactions with the environment are involved.

Climatic Conditions. Plant response to ozone and PAN may be altered by climatic conditions before, during, and after exposure.[210] Plants may be sensitized to a given set of conditions after 1-5 days. It appears that the major conditioning occurs within the first 3 days under a given set of environmental conditions. Conditions during exposure may be critical, and conditions after exposure are probably important, but less so than those before and during exposure. The responses of plants to ozone and PAN under varied climatic conditions are studied primarily under laboratory and greenhouse conditions, but field observations have often substantiated the results. Most studies have involved individual climatic factors and one or two response measures, usually including injury. Several have dealt with environmental interactions. Sufficient information exists for generalizations of plant response, but there is much uncertainty, because of the small number of species studied and the lack of information on the interactions of factors.

- Light quality: The quality of light affects the growth and development of plants and plays some role in determining the response of pinto bean to PAN.[134,136] Injury to pinto bean from PAN was maximal when exposed at 420 and 480 nm and less than half that at 640 nm. The response is apparently associated with changes in carotenoid pigments. Shinohara et al.[487] reported

an effect on tobacco (H-mutant) when exposed at 0.40-ppm ozone for 30 min. They found the least injury in far red, followed by blue, green, and white, with the greatest effect in red. This work was preliminary. More information is needed on the effects of light quality.

• Photoperiod: The effect of a given light period within a 24-h cycle exerts physiologic control over some aspects of plant development. Research has shown that plants are more sensitive to ambient oxidants and ozone when grown under an 8-h photoperiod than either a 12-h or a 16-h photoperiod [212, 268, 320] (Table 11-8).

• Light intensity: The intensity of light affects many physiologic processes within plants and is known to affect the response of plants to oxidant pollutants. Studies have been reported on effects of intensity before, during, and after exposure (Table 11-9).

Dugger et al.[136] found a direct correlation between the sensitivity of pinto bean to PAN and increasing light intensity. Pinto bean required light just before, during, and after a PAN exposure for injury to occur.[136, 513] This was not true with exposure to ozone, although some light period was necessary.

Generally, plants are more sensitive to ozone when grown at lower light intensities. This was shown for pinto bean[137, 138, 212] and tobacco,[137, 212, 487] but was reversed in tobacco Bel W .[138, 3] The reason for this reversal was unclear. In both pinto bean and tobacco, there was greater injury from ozone when plants were grown at lower light intensities when the humidity was 60% or 80%.[137] Dugger et al.[136] found greater injury in plants grown at 900 ft-c (about 9,690 luces) than at 2,200 ft-c (about 23,680 lx), but Dunning and Heck[138] reported no difference between 1,000 and 2,000 ft-c (about 10,760 and 21,530 lx) for either pinto bean or tobacco exposed

Table 11-8

Response of Plants to Ozone, as Conditioned by Photoperiod

Plant Species	Ozone Concentration, ppm, h	Notes	Response, % injury Photoperiod, h 8	12	16	Reference
Tobaco, cultivar White Gold	0.67, 5	--	57	40	27	320
Tobacco, cultivar Bel W_3	0.30, 1	Control conditions, 2,000 ft-c (21,529 lx)	54	--	19	212
Bean, cultivar Pinto	0.30, 1	Control conditions, 2,000 ft-c (21,529 lx)	71	--	18	212

Table 11-9

Response of Plants to Ozone, as Conditioned by Growth and Exposure Light Intensity

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Growth or Exposure[b]	Injury, %, at Light Intensity, ft-c (lx)				Reference
Bean, cultivar Pinto	0.35, 1	Grown under control conditions; ET,85F (29C)		0 (0)	100 (1,076)	1,800 (19,375)	2,600 (27,987)	216
			Exposure	2	10	73	89	
Bean, cultivar Pinto	0.30, 1	Control conditions; PP, 8 h		2,000 (21,529)		3,000 (32,293)		212
			Growth	71		49		
Tobacco, cultivar Bel W_3	0.30, 1	Control conditions; PP, 8 h	Growth	54		41		212
Bean, cultivar Pinto	0.40, 1	Control conditions; PP, 8 h; EL, 2,000 ft-c (21,530 lx)		1,000 (10,764)	2,000 (21,529)	4,000 (43,057)	6,000 (64,585)	138
			Growth	80	84	28	--	
	0.40, 1	Control conditions; PP, 8 h; GL, 4,000 ft-c (43,057 lx)	Exposure	16	28	57	67	
Tobacco, cultivar Bel W_3	0.40, 1	Control conditions; PP, 8 h; EL, 4,000 ft-c (43,057 lx)	Growth	21	19	38	--	138
	0.40, 1	Control conditions; PP, 8 h; GL, 4,000 ft-c (43,057 lx)	Exposure	8	27	38	32	
Bean, cultivar Pinto	0.40, 1	Control conditions; PP, 8 h	Growth at 80% RH	--	78	44	--	137
			at 60% RH	--	66	47	--	
	0.40, 1	Control conditions; PP, 8 h	Exposure	--	50	68	--	

Table 11-9 (Cont.)

Response of Plants to Ozone, as Conditioned by Growth and Exposure Light Intensity

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Growth or Exposure[b]	Injury, %, at Light Intensity, ft-c (lx)				Reference
Tobacco, cultivar Bel W_3	0.40, 1	Control conditions; PP, 8 h	Growth at 80% RH	--	36	27	--	137
			at 60% RH	--	43	34	--	
	0.40, 1	Control conditions; PP, 8 h	Exposure	--	33	35	--	

[a] PP = photoperiod, ET = exposure temperature, EL = exposure light, GL = growth light.

[b] Time of light treatment.

to ozone. Dugger et al.[136] found a relation between plant age and light intensity. Peak sensitivity to ozone was about the same at both intensities, but the span of plant age that was sensitive was increased at 900 ft-c (about 9,690 lx). This could have an important bearing on environmental work and has not been seriously considered by other workers.

Sensitivity to ozone as light changes during exposure generally increases with intensity. This was found for tobacco[138] between 1,000 and 4,000 ft-c (about 10,760 and 43,060 lx), with a slight decrease at 6,000 ft-c (about 64,580 lx), and for pinto bean[137,216] between 1,000 and 6,000 ft-c (about 10,760 and 64,580 lx). When pinto bean was grown at 4,000 ft-c (43,060 lx), there was an increase in sensitivity from 1,000 to 6,000 ft-c (10,760 and 64,580 lx) during ozone exposure; but, at lower intensity during growth, the 4,000- and 6,000-ft-c (43,060- and 64,580-lx) exposure intensity gave similar responses.[138] Heck[137] reported that exposure light had no effect on the response of tobacco or pinto bean to ozone when the relative humidity was 80%, but had an effect at 60%.

Shinohara et al.[487] reported that up to 10 h of postexposure light did not affect the response of tobacco (H-mutant) to ozone.[113] Davis and Wood delayed symptom development in Virginia pine when the plants were held in the dark for extended periods after ozone exposure. However, the final severity of response was unchanged.

- Temperature: Variations in plant response to ozone or oxidants occur with increasing growth temperature, if a given temperature is maintained for 3 days or more before exposure.[320] Macdowall found that a low day or high night temperature increased the susceptibility of White Gold tobacco to ozone. He reported no interaction between day and night temperatures.

Juhren et al.[268] used eight combinations of day and night growth temperatures with Poa annua and then exposed these plants to ambient oxidants for a day. The sensitivity varied with plant age, showed a partial reversal after 3 days of changed temperatures, and was greatest at the 26 C-20 C day-night temperatures. Radish[4,417] and pinto bean[139] were more sensitive if grown under cooler conditions, whereas snap bean,[7] soybean,[139] pinto bean,[138,139] Bel W 3 tobacco,[138,363,485] Virginia pine,[107,113] and white ash[589] were more sensitive if grown under warmer conditions.

Shinohara et al.[486] looked at growth, exposure and postexposure temperatures, and their effect on the response of several tobacco cultivars to ozone. Plants were grown at constant temperature (day/night) during growth and were kept in the dark for 48 h after exposure. These elements of design weaken the value of this report. However, generally they showed an interaction between growth and postexposure temperature, night temperatures were more important than day temperatures, and lower temperatures during exposure increased the sensitivity of tobacco to ozone.

Drummond[128] reported increased injury to petunia (cultivar White Cascade) with increasing temperature when exposed for 1 h to PAN at 0.15 ppm.

Early reports suggested a positive correlation between plant response to ozone and increasing exposure temperature, to about 30 C. These results were shown for pinto bean when exposures were under greenhouse conditions,[216] but an inverse correlation was found when they were under controlled lighting in growth chambers. This suggested that, under greenhouse conditions, it was not possible to separate light and temperature effects. This inverse relationship has been shown for Bel W 3 tobacco,[64,65,138,216] Lemna minor,[159]

Virginia pine,[107,113] and white ash.[589] Under some conditions, this does not seem to hold for pinto bean,[138,139] in which a direct correlation was found from 20 to 32 C.

It has been suggested[210] that higher postexposure temperatures will cause an increase in sensitivity.[107,113] This was shown for Virginia pine[589] and for white ash,[589] but the reverse occurred in Bel W tobacco[485] and in radish.[4,3]

Two papers have considered interactions between temperature and other variables. Dunning et al.[139] studied possible interactions among species (Dare soybean and pinto bean), ozone dose, potassium nutrition, growth temperature, and exposure temperature. The two species did not respond the same, except that there were no interactions with potassium nutrition in either species. Soybean showed no interactions between dose and temperature under either temperature design. Pinto bean showed strong interactions between dose and growth temperature and between exposure and growth temperatures and a mild interaction between dose and exposure temperature, with no higher-order interactions. The dose-growth temperature interaction is shown graphically in Figure 11-2. Apparently, pinto bean is very sensitive to a variety of stresses, and these variations make pinto bean both a unique test plant and one that can be very difficult to handle. In later studies, Dunning and Heck[138] looked at interactions among growth temperature, exposure temperature, and plant species (Bel W tobacco[3] and pinto bean). They found that the two species responded differently to the temperature conditions. Pinto bean showed no interaction between growth and exposure temperatures, whereas tobacco showed a strong interaction. For tobacco, growth temperature had little effect at an exposure

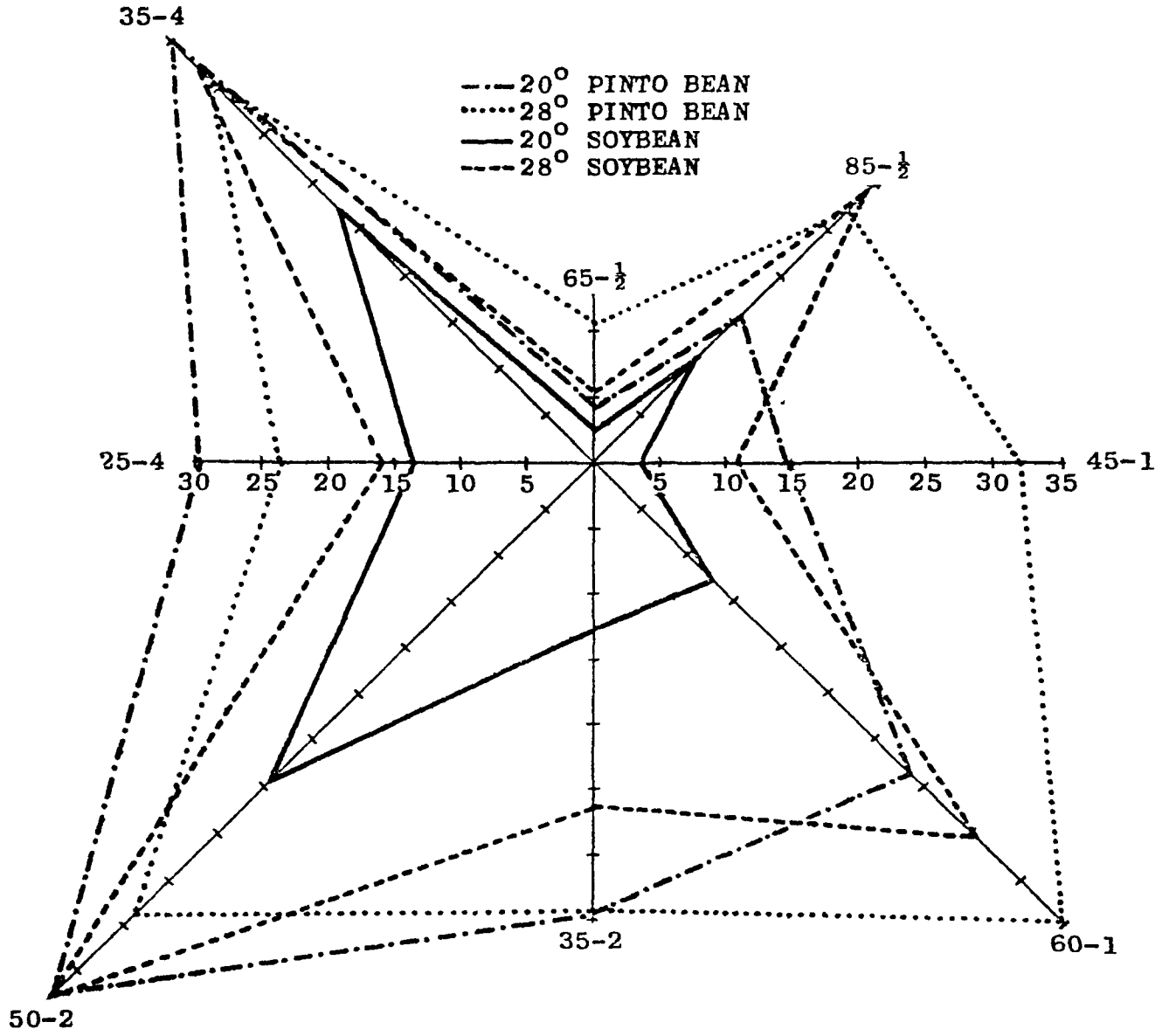


Figure 11-2. The significant interaction of growth temperature in C, ozone dose (concentration x time), and species on foliar response (% injury) to ozone. Dose is shown at the end of each of the eight axes as concentration (parts per hundred million) and time (hours). Percentage injury is shown in 5% increments along each axis. Each value is a mean of 15 observations. The LSD at the 1% level is 8.3 for the injury response. This value can be used to compare any two points in the figure. (Reprinted with permission from Dunning et al.[139])

temperature of 16 or 32 C, but a marked effect at 21 or 27 C (sensitivity increased with increasing growth temperature). These types of interactions need further clarification as we attempt to understand the effects of a total environmental system on the response of plants to oxidant pollutants.

Crops that show greater injury under cooler conditions may be more severely injured in early spring (assuming that oxidant is present). Feder and Sullivan[159] used duckweed to remove the confounding effects of stomatal movement. They reported similar results in light and dark fumigations with temperature variations. Dunning et al.[139] found no changes in carbohydrate content associated with sensitivity of either pinto bean or Dare soybean. This was also reported for radish,[4] except that cultivar Cavalier showed an increase in carbohydrate after an ozone exposure in a single experiment.

A summary of these temperature effects is found in Table 11-10.

- Relative humidity: Field observations suggest that plants are more sensitive to oxidants as relative humidity increases. Davis and Wood[107,113] found an increase in sensitivity of Virginia pine to ozone at a high humidity during exposure, but reported no effects from growth or postexposure humidity changes. Otto and Daines[424] found similar results over a wider humidity range and over several ozone concentrations during exposure of pinto bean and Bel W 3 tobacco. They did not study growth or postexposure conditions. Dunning and Heck[137] showed a significant increase in the response of pinto bean, but not of tobacco, to increased exposure humidity. For growth humidity, tobacco sensitivity decreased with an increase in humidity; pinto bean response was unaffected by humidity at a growth light of 4,000 ft-c (43,060 lx), but was increased with an increased humidity at 2,000-ft-c (21,530-lx) growth light. In later work,[138] Dunning

Table 11-10

Response of Plants to Ozone, as Conditioned by Growth or Exposure Temperature

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Type of Response	Growth or Exposure[b]	Response, % reduction from control Day Temp.[c] or Day/Night Temp., C	Reference
Tobacco, cultivar White Gold	0.67,5	--	% injury	Growth	15.6 / 23.9 / 32.2	320
				day	-- / 44 / 27	
				night	28 / 33 / 44	
Radish, cultivar Cavalier	0.24, 4	PP, 16 h	% growth red.	Growth	20/15 / 30/25	417
				60 mg N/1	50 / 43	
				300 mg N/1	55 / 47	
Bean, cultivar Blue Lake	0.50, 2	Exposed twice	% chl. red.	Growth	20/15: 7; 25/20: 29; 30/25: 55	7
			% red.1st. trif.dry wt		14 / 32 / 33	
			% red.root dry wt		27 / 31 / 28	
Tobacco, cultivar Bel W_3	0.20, 4	PP, 12 h; 2,000 ft-c (21,529 lx); 85% RH	% injury	Exposure	10: 66; 15.6: 34; 21.1: 13; 26.7: 11; 32.2: 1	64,65
Lemna minor, L.	0.25, 4	PP, 8 h; 500 ft-c (5,382 lx)	% red.chl.	Exposure	7.2: 53; 18.3: 18; 25: 0; 30.6: 0	159
Tobacco, cultivar Bel W_3	3 (ambient oxidant exp; ave., 0.69 ppm-h)	--	% injury	Post-exposure	10: 50; 13.9: 33; 20: 10; 23.9: 0	485
Tobacco, cultivar H-2	0.35, 2	Constant temp. 4 days before exposure (23 C), 48 h dark at 13 C after exposure	% injury (5 leaves/plant affected)	Exposure	13: 66; 20: 50; 27: 9; 32: 0	486

Table 11-10 (Cont.)

Response of Plants to Ozone, as Conditioned by Growth or Exposure Temperature

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Type of Response	Growth or Exposure	Response: Day Temp. of Day/night Temp., C	Reference
Radish, cultivar Cherry Belle	0.25, 3	25/20 C normal temp.; PP, 16 h; 1,800 ft-c (19,375 lx); 82% RH	% growth red. of roots	15 days growth	20/15: 37; 30/25: 25	4
				15 days post-exposure	52; 38	
Soybean, cultivar Dare	8 doses	PP, 12 h; 2,200 ft-c (23,681 lx); 1 wk at special temp. for growth	% injury	Growth	20: 14; 28: 28	139
Bean, cultivar Pinto	8 doses	PP, 12 h; 2,200 ft-c (23,681 lx); 1 wk at special temp. for growth	% injury	Growth at 20 ET	28 24	139
				Growth at 28 ET	30 43	
				Exposure at 28 GT	24 43	
Poa annua	Ambient Oxidant-24	Controlled growth	% injury	Growth	20/14: 14; 23/17: 11; 26/20: 20; 30/24: 1	268
Tobacco, cultivar Bel W_3	0.21, 2	2 wk of conditioning	% injury	Growth	20/15: 7; 25/20: 39	363
Bean, cultivar Pinto	0.55, 1	Greenhouse exposure (correlated with light intensity)	% injury	Exposure	22.2: 25; 26.7: 54; 32.2: 77	216

Table 11-10 (Cont.)

Response of Plants to Ozone, as Conditioned by Growth or Exposure Temperature

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Type of Response	Growth or Exposure	Response: Day Temp. of Day/night Temp., C	Reference
Bean, cultivar Pinto	0.35, 1	Grown under control conditions; exposed under constant conditions	% injury	Exposure	18.3: -- ; 23.9: 77 ; 29.4: 68 ; 35: 51	216
	0.32, 1	Grown under control conditions; exposed under constant conditions	% injury	Exposure	18.3: 77 ; 23.9: 68 ; 29.4: 56 ; 35: 41	
Tobacco, cultivar Bel W_3	0.35, 1	Grown under control conditions; exposed under constant conditions	% injury	Exposure	18.3: -- ; 23.9: 61 ; 29.4: 44 ; 35: 29	216
Pine, Virginia	0.50, 1.5	Control conditions; 3-yr seedlings	% injury	Exposure	10: 52 ; 15: 33 ; 21: 27 ; 27: 15 ; 32: 3	113
	0.25,4	Control conditions; 3-yr seedlings	% injury	Growth	10: -- ; 15: 3 ; 21: -- ; 27: -- ; 32: 42	
				Post-exposure	10: -- ; 15: 19 ; 21: -- ; 27: -- ; 32: 34	
Ash, white	0.25,4	Control conditions; 1-yr seedlings	% injury	Growth	16: 49 ; 27: 66	589
				Exposure	16: 67 ; 27: 51	
				Post-exposure	16: 54 ; 27: 63	
Bean, cultivar Pinto	0.40, 1	Control conditions; PP, 8 h; ET, 27	% injury	Growth	16: -- ; 21: 39 ; 27: 57 ; 32: 76	138
	0.40, 1	Control conditions; PP, 8 h; GT, 27	% injury	Exposure	16: 41 ; 21: 32 ; 27: 57 ; 32: 89	

Table 11-10 (Cont.)

Response of Plants to Ozone, as Conditioned by Growth or Exposure Temperature

Plant Species	Ozone Concentration, ppm, h	Notes	Type of Response	Growth or Exposure	Response: Day Temp of Day/night Temp, C				Reference
					16	21	27	32	
Tobacco, cultivar Bel W_3	0.40, 1	Control conditions; PP, 8 h; ET, 27	% injury	Growth	--	31	42	61	138
		Control conditions; PP, 8 h; GT, 27	% injury	Exposure	58	44	42	11	

[a]PP = photoperiod, ET = exposure temperature, GT = growth temperature.

[b]Time of temperature treatment.

[c]Temperatures are shown as day or as day/night values and are underlined.

and Heck found an increase in sensitivity of both tobacco and pinto bean to ozone with increasing exposure humidity from 45 to 90%, regardless of growth humidity. However, there was an interaction between growth humidity and exposure humidity such that the response of these two species to growth humidity was conditioned by the exposure humidity. Plants were always more sensitive when grown at 75% humidity, regardless of the exposure humidity. Table 11-11 shows some of these results.

- Carbon dioxide: Stomatal activity is affected by carbon dioxide, and it may affect plant sensitivity to oxidants. Heck and Dunning[212] reported a decrease in sensitivity of tobacco to ozone if the tobacco was exposed to added carbon dioxide at 500 ppm immediately before and during exposure to ozone (22% injury with added carbon dioxide, and 66% injury without). If this is a general plant response, the carbon dioxide now added to greenhouses to increase productivity may also increase resistance of the plants to oxidant pollutants.

- Field observations: Canadian workers using ambient oxidant dose have correlated meteorologic variables with plant injury.[324, 396] A correlation was discovered when an empirical relationship involving evapotranspiration (the coefficient of evaporation) was developed and used to modify the dose information. This empirical relationship has been used on a limited basis to predict damaging oxidant concentrations from monitored meteorologic conditions.

Linzon[313] reported SNB symptoms on white pine after several days of wet weather followed by a continuous sunny period. Symptoms were noted several times during the 1957 - 1964 growing seasons at Chalk River, Ontario, Ontario, but time of occurrence did not correlate well with peak oxidant

Table 11-11

Response of Plants to Ozone, as Conditioned by Growth and Exposure Humidity

Plant Species	Ozone Concentration, ppm, h	Notes[a]	Growth or Exposure[b]	Response, % Injury (% RH)[c]	Reference
Pine, Virginia	0.25, 4	Control conditions; 3-yr seedlings		60 85	113
			Exposure	4 25	
			Growth	50 58	
	0.25, 4	Juvenile	Exposure	1 35	
Bean, cultivar Pinto, and Tobacco, cultivar Bel W_3, averaged	0.40, 1	Control conditions; PP, 8 h		45 60 75 90	138
			Growth		
			45% EH	36 39 41 31	
			90% EH	73 67 81 80	
		Control conditions; PP, 8 h; 75% GH	Exposure	41 53 70 81	
Bean, cultivar Pinto	0.40, 1	Control conditions; PP, 8 h; 2,000 ft-c (21,529 lx)		60 80	137
			Growth	66 78	
		Control conditions; PP, 8 h	Exposure	52 67	
Tobacco, cultivar Bel W_3	0.40, 1	Control conditions; PP, 8 h; 2,000 ft-c (21,529 lx)	Growth	42 36	137
		Control conditions; PP, 8 h	Exposure	33 36	
Tobacco, cultivar Bel W_3	0.30, 1.5	Control conditions; 31 C		26 51 95	424
			Exposure	9 39 50	
Bean, cultivar Pinto	0.20, 1.5	Control conditions; 31 C	Exposure	0 0 55	424

Table 11-11 (Cont.)

Response of Plants to Ozone, as Conditioned by Growth and Exposure Humidity

Plant Species	Ozone Concentration ppm, h	Notes[a]	Growth or Exposure[b]	Response % Injury (% RH)[c]	Reference
Ash, white	0.25,4	Controlled conditions; 1-yr seedlings		60 80	589
			Growth	33 46	
			Exposure	38 41	
			Post-exposure	36 41	

[a] PP = photoperiod, GH = growth humidity, EH = exposure humidity.

[b] Time of humidity treatment.

[c] Humidity values are underlined.

concentrations.[314] Berry and Ripperton[29] observed emergence tipburn on susceptible trees in West Virginia several days after oxidant peaks of 0.065 ppm. They found that container-grown susceptible pine clones were protected from injury if placed in a chamber supplied with charcoal-filtered air.

Such factors as windspeed and barometric pressure appear to play little or no role in affecting plant sensitivity to oxidant pollutants. Air movement would be expected to play a role under ambient conditions, because of its known effect on leaf boundary layers, but it probably has little effect in chamber work, unless wind velocities are greater than 1 mph (1.6 km/h).[45,207,232]

It is widely believed that vegetation growing in the humid eastern United States would be severely injured, if oxidant concentrations reached the daily peak concentrations (0.20-0.40 ppm) commonly experienced in the less humid sections of California. An air pollution episode that occurred on July 27-30, 1970, in the Washington, D.C., area is indicative of what may happen. During this 4-day period, the peak oxidant concentrations ranged from 0.14 to 0.22 ppm and were accompanied by a low concentration of sulfur dioxide (0.04 ppm). Oxidant injury was observed on 31 tree, 15 shrub, and 18 herbaceous species in an area of 72 mi^2 (about 187 km^2). Increased emission of the precursors of photochemical oxidant formation could result in repeated episodes of acute injury or even chronic injury to eastern vegetation.

Several workers have explored the relationships between ozone effects on vegetation and several climatic factors. Thus, we have some understanding of how these factors may affect a plant's response to ozone and probably to ambient oxidants.[137-139] The few reports on these interactions suggest that much remains to be learned about how these climatic

factors interact in causing a plant response. Studies to date suggest that stresses applied during exposure have their greatest effect on stomatal activity, whereas those applied during growth affect a physiologic basis of resistance that is not related to stomatal function. Postexposure reactions would no doubt be physiologic. Interactions between growth and exposure conditions would combine these stress factors. A physiologic stress may be so pronounced that the normally responsive stomatal mechanism would not function during exposure.[137] More emphasis needs to be placed on the study of multiple-stress interactions.

Edaphic Conditions. Soil water stress during growth and exposure to oxidant is probably the most significant environmental factor affecting plant response. Khatamian et al.[280] found less reduction in chlorophyll content and an increase in dry-matter production in tomato exposed to ozone at 0.50 or 1.00 ppm for an hour, if plants were grown under a water stress that did not itself cause a reduction in growth. Plants in the three-leaf stage were most sensitive to ozone if grown in optimal soil moisture (Table 11-12). Markowski and Grzesiak[348] found protection from ozone in bean and barley grown under drought conditions. Several early field studies[117, 504, 577] showed a close correlation between soil moisture and oxidant injury to several tobacco cultivars. Field observations generally suggest that sensitive plants may become resistant under drought conditions. It is possible that drought during growth causes physiologic changes within the plant that increase resistance, whereas similar conditions during exposure may reduce stomatal opening. Adedipe et al.[8] reported some stomatal closure in tomato exposed to ozone at 1.00 ppm for 1.5 h. However, closure may have been triggered by the low light intensity of exposure (50 ft-c, or about 540 lx). Rich and

Table 11-12

Effects of Soil Moisture on Response of Selected Plants to Oxidant Stress

Plant Species	Oxidant Concentration, ppm, h	Type of Response	Response, % Reduction from Control Moisture Conditions (high → low)[a]			Reference
Tobacco, cultivar Catterton	Ambient oxidant	% injury	Irrigated 29	Normal 11		360
Tomato, cultivar Fireball			90% turgid	80% turgid		
	1.00, 1.5	% red. in chlorophyll	54	10		8
	1.00, 1.0	% red. in chlorophyll	67	24		280
	0.50, 1.0	% red. in chlorophyll	36	+3		280
	1.00, 1.0	% red. leaf dry wt	48	+40		280
Beet, garden			-40 kPa	-440 kPa	-840 kPa	412
	Control	% red. in dry wt of storage root from nonsaline control	0	24	68	
	0.20, 3 (daily for 38 days)		40	52	69	
Bean, cultivar Pinto			-40 kPa	-200 kPa	-400 kPa	244
	Control	% red. in shoot dry wt from nonsaline control	0	18	78	
	0.15, 2/day (63 days)		27	42	87	
	0.25, 2/day (63 days)		93	91	88	
	Control	% red. in root dry wt from nonsaline control	0	25	65	
	0.15, 2/day (63 days)		25	28	78	
	0.25, 2/day (63 days)		91	89	79	

[a]Special soil moisture conditions are underlined. kPa = kilopascals (bars x 10^2).

Turner[457] found rapid stomatal closure in pinto bean during a 30-min exposure to ozone at 0.20-0.25 ppm, if plants were grown under a soil moisture stress. Closure under optimal water availability was slower. They also reported rapid closure in pinto bean and Bel W_3 tobacco if the plants were conditioned and exposed to ozone at a relative humidity of 37%; this was not found at 73%. Their evidence suggests that ozone may induce more rapid closure when plants are already under some type of water stress.

Ting and Dugger[530] related activitivity of two tobacco cultivars to the size of the root systems; those with larger root systems were more sensitive. Several workers have recommended withholding water from greehouse and irrigated crops during times of high pollution potential.

Starkey[495] found that a sensitive bean exposed to subacute concentrations of PAN was sensitive to postexposure drought, but more resistant plants were not. This was related to effects of PAN on membranes.

In perennial vegetation, adequate soil moisture in the early season interacts to allow open stomata, and thus oxidant injury occurs. When soil moisture becomes limiting, the nonfunctional stomata of injured leaves may remain open, thus increasing transpiration. The moisture stress induced in the late season is additive with oxidant injury, because both increase defoliation.

Oertli[411] found that both salinity and low soil moisture increased the resistance of sunflower to ambient oxidants. He used four different strength salt solutions from 0.75 to 6 atm and two soil moisture levels (0.3 and 0.8 atm). Ogata and Maas[412] exposed garden beet for 38 days to ozone at 0.20 ppm and varied exposure time from 0 to 3 h/day. The garden beet was protected from ozone injury and yield reductions at high soil salinity,

but this protection was offset by growth reductions caused by the salinity (Table 11-12). The storage root was the most sensitive yield indicator for both salinity and ozone stress. They found that yield was not affected until the daily ozone exposure was 2 h (-40 kPa) or 3 h (-440 kPa). Ozone had no effect at -840 kPa. Hoffman et al.[244] reported similar results with pinto bean exposed to four ozone concentrations for 2 h/day from seed to harvest. They had three degrees of nutrient osmotic potential and used sodium chloride to increase salinity. The 0.15-ppm treatment at -40 kPa gave a 46% reduction in bean fresh weight, whereas no yield was found at 0.25 ppm and above. Results for top and root dry weights were similar (Table 11-12). Maas et al.[319] studied the response of pinto bean to ozone and salinity as conditioned by concentration and duration of exposure (Table 11-13). They found the injury and growth reductions were sigmoidal at the low salinity (Tables 11-3, 11-5, and 11-13). Salinity suppressed plant growth, but extended the ozone tolerance for both growth and injury response. In this study, the roots were more severely affected than the tops. However, in contrast with their earlier study,[244] the pinto beans were exposed for only 14 days and harvested as young plants. Maas et al.[319] also looked at nutritional effects; although some redistribution of nutrient was found (with respect to calcium, magnesium, potassium, and nitrogen), no serious nutritional imbalances occurred. In the last of a series of excellent papers on salinity effects, Hoffman et al.[245] studied the effects of four ozone concentrations (2 h/day for 21 days) at four levels of salinity on biomass production in alfalfa (Table 11-14). As in the earlier studies, the ozone effect decreased with increasing salinity. However, in contrast, they found that -200 kPa gave protection from ozone

Table 11-13

The Interaction of Ozone and Salinity on Biomass of Pinto Bean Exposed to Ozone[a]

Osmotic Potential, kPa	Ozone Concentration, ppm	Reduction from Control Weight of Plants Grown at -40 kPa and Not Exposed to Ozone, %							
		Dry Wt of Leaves				Dry Wt of Roots			
		0	0.30	0.60	0.90	0	0.30	0.60	0.90
		(ozone dose, ppm-h)[b]				(ozone dose, ppm-h)			
-40	0.15	0	14	28	67	0	18	53	72
	0.30	0	36	68	75	0	64	83	87
-240	0.15	25	31	41	50	24	25	35	51
	0.30	25	39	70	76	24	50	81	85
-440	0.15	59	65	60	67	47	49	40	53
	0.30	59	55	63	75	47	42	56	74

319

[a]Data from Maas et al.

[b]Specific dose was given daily for 14 days; the duration of exposure can be determined from ozone concentrations used.

Table 11-14

Interaction of Ozone and Salinity on Top Growth of Alfalfa[a]

Ozone Concentration, ppm[b]	Reduction from Control Weight of Plants Grown at -40 kPa and Not Exposed to Ozone,%[c]			
	-40 kPa	-200 kPa	-400 kPa	-600 kPa
0	0	+2	24	35
10	16	11	29	41
15	26	14	33	49
20	39	23	31	48

245

[a] Data from Hoffman et al.

[b] Concentration shown was given 2 h/day for 21 days.

[c] Values that differ by 10 are probably significantly different.

with no effect of salinity on biomass production. This suggests that moderate salinity, in areas of high oxidant, could protect forage legumes (such as alfalfa) from yield reductions. They also found that both high salinity and ozone increased water use efficiency of alfalfa and leaf diffusion resistance. These reports on salinity suggest that we are dealing with a moisture stress problem. Although this may involve stomatal response, it is more likely that we are dealing with a changed physiologic state that increases the resistance of the plants to ozone effects. At high salinity, both phenomena may play a role, but salinity itself can cause major yield reductions in the crops reported on here.

The importance of soil fertility in the response of plants to oxidants is not understood. No research has attempted to explore, in depth, the nutritional interactions with oxidants. Nitrogen nutrition has received the most attention. Menser and Street,[360] in an ambient-oxidant study, reported an increase in tobacco fleck with increasing application of soil nitrogen. This was also found in spinach exposed to ozone.[54] Leone et al.[311] reported that nitrogen concentrations optimal for growth produced the most sensitive tobacco, whereas either higher or lower nitrogen applications increased resistance to ozone. The opposite was reported for White Gold tobacco, in which the optimal nitrogen concentration for growth gave the greatest resistance to ozone.[320] Ormrod et al.[417] found no effect of two nitrogen concentrations on growth of radish exposed to ozone. It is apparent that nitrogen concentrations and other conditions used in these experiments were not sufficiently critical for an evaluation of nitrogen-oxidant interactions. Craker[90] reported that increased nitrogen in the nutrient increased chlorophyll loss in duckweed exposed to ozone. Several studies have explored the importance of phosphorus

in controlling the sensitivity of plants to oxidants. Work by Ormrod et al.[417] suggested that radish grown at 20 C is more sensitive to ozone at a higher phosphorus concentration, but that phosphorus has no effect at a growth temperature of 30 C. Ripaldi and Brennan[464] reported an increase in tissue phosphorus for pinto bean after exposure to ozone. They did not determine nutrient phosphorus. Leone and Brennan[308] reported an increased ozone injury to tomato with increasing nutrient and tissue phosphorus (1.5 - 62 ppm). Brewer et al.[54] found an interaction between potassium and phosphorus in the response of spinach to ozone. They reported that, at low phosphorus, an increase in potassium tends to increase injury, but, at high phosphorus, the potassium increase tends to reduce injury. Craker[90] reported a similar effect on duckweed; chlorophyll loss increased with increasing potassium at a medium phosphorus content, but decreased with increasing potassium at zero or higher phosphorus content. There is an apparent interaction between these nutrients, but its importance is not known. Dunning et al.[139] found that pinto bean and soybean were more sensitive to ozone at low nutrient potassium, whereas Adedipe et al.[7] reported that low nutrient sulfur increased the response of Blue Lake snapbeans to ozone. McIlveen et al.[351] reported increased injury with increasing soil zinc. Craker[90] found no change in the response of duckweed to ozone when total salt was varied from one-tenth to one-half of a full-strength nutrient solution. The response in a full-strength nutrient was about one-third less. Soil nutrition probably plays a fairly important role in plant response to pollutants only in cases of nutrient imbalances, although total salts should affect response under some conditions. In soils with balanced fertilizer, plants may respond fairly uniformly to oxidant stress. Table 11-15 summarizes several nutritional studies.

Table 11-15

Effects of Various Nutrients on Response of Selected Plants to Ozone (Oxidant) Stress

Plant Species	Oxidant Concentration, ppm, h	Type of Response	Element	Response, % Reduction from Control — Nutritional Levels (low → high)[a]				Reference
Tobacco, cultivar White Gold	0.35, 48	% injury	Nitrogen	0.1 21	0.5 16	1.0 4	5.0[b] 22	320
Tobacco, cultivar Catterton	Ambient Oxidant	% injury	Nitrogen	60 lb/acre 29		90 20	120 11	360
Tomato, cultivar Rutgers	0.18, 4	% injury	Nitrogen	28 mg/l 20	280 40	560 40	1120 20	311
Spinach, cultivar Viroflay	0.25, 9/day, 3 days	% injury	Nitrogen	10 ppm 3	30 ppm 18	90 ppm 22		54
Radish, cultivar Cavalier	0.25, 4	% growth red.	Nitrogen	60 mg/l 50	300 mg/l 55			417
Lemna minor, L. (Duckweed)	0.35, 2	% chl. red.	Nitrogen	0 18	0.9 - 3.6 mM 28-42		4.5 mM 55	90
Tomato, cultivar Rutgers	0.15, 3	% injury	Phosphorus	1.5 ppm 0	15.5 ppm 0	62 ppm 40		311
	0.25, 3	% injury	Phosphorus	20	40	60		
	0.30, 3	% injury	Phosphorus	20	60	60		
Spinach, cultivar Viroflay	0.25, 9/day, 3 days	% injury	Phosphorus	20 ppm	150 ppm			54
			5 ppm K	14	13			
			40 ppm K	25	9			
Radish, cultivar Cavalier	0.25, 4	% growth red.	Phosphorus	30 mg/l 35	150 mg/l 43			417

Table 11-15 (Cont.)

Effects of Various Nutrients on Response of Selected Plants to Ozone (Oxidant) Stress

Plant Species	Oxidant Concentration, ppm, h	Type of Response	Element	Response, % Reduction from Control — Nutritional Levels (low → high)[a]			Reference
Lemna minor, L. (Duckweed)	0.35, 2	% chl. red.	Phosphorus	0 24	0.33 34	0.66[b] 41	90
Bean, cultivar Blue Lake	0.50, 2/day 2 days	% chl. red.	Sulfur	1.3 mg/l 55		32 mg/l 11	7
Soybean, cultivar Dare	8 doses (acute)	% injury	Potassium	105 mg/l 40		710 mg/l 23	139
Bean, cultivar Pinto	8 doses (acute)	% injury	Potassium	26		18	139

[a] Nutritional values are underlined and show a number of different units.

[b] Relative to a full-strength nutrient solution.

Stolzy et al.[498] found that low oxygen partial pressures over soil (0-10 mm Hg) for 40 h effectively protected tomato from injury by ozone (0.35 ppm for 3 h). These anaerobic conditions also decreased water use and general vigor. In later work,[497] Stolzy et al. reported that, with anaerobic soil conditions, the photosynthetic rate stablized within 24 h, well below the control (32%). Exposure to ozone (at 0.17 ppm for 3 h) caused no injury, but caused a marked reduction in the photosynthetic rate of the control with a slight increase in photosynthetic rates in plants under anaerobic conditions. They also showed that a 3-h period of anaerobic treatment of the soil affected the plant response to an ozone exposure 22 h later. The anaerobic treatment showed less injury and the photosynthetic rate was reduced less than in the control plants. These changes were related to carbohydrate changes within the plants. These experiments suggest that even passing periods of low soil oxygen tension could reduce plant response to ozone stress.

Blum and Tingey[34] found that ozone reduction in soybean root growth and nodulation was a function of foliar impact. They found no direct effect of ozone on the roots or soil systems surrounding the roots.

Pollutant interactions. Oxidant air pollutants exist as parts of a complex mixture of gases, many of which may be phytotoxic. However, except for ambient air studies and simulated photochemical oxidant studies, little research was done with pollutant combinations until the classic work of Menser and Heggestad in 1966.[357] It is of interest that Thomas et al.[517] suggested that sulfur dioxide might lessen the effect of oxidants in causing foliar injury to pinto bean. Middleton et al.,[368] working with ratios of sulfur dioxide to ozone of from 4:1 to 6:1, did not observe an increase in

injury, although hey found that, at 4:1, ozone appeared to interfere with the expected sulfur dioxide injury. Heck[209] found that various combinations of ethylene, propylene, and acetylene, when mixed with products of irradiated propylene-nitrogen dioxide mixtures, did not decrease or increase the development of foliar injury.

Menser and Heggestad[357] first reported that exposure to mixtures of sulfur dioxide (0.50 ppm) and ozone (0.03 ppm) for 2 or 4 h caused 23-48% foliar injury to the sensitive tobacco cultivar Bel W$_3$, whereas the concentrations of the individual gases produced no injury. This study stimulated plant scientists to develop research on pollutant combinations.

Reinert et al.[447] have suggested the use of the terms "simultaneous" (mixtures of pollutants), "sequential" (one pollutant followed by a second pollutant), and "intermittent" (when there is some period between sequential exposures) to refer to exposures in discussing studies of pollutant combinations. They also recommended terminology for use in describing plant response to pollutant combinations: less than additive, -; additive, 0; and greater than additive, +.

Ozone and sulfur dioxide mixtures are of special interest, owing to their widespread occurrence and to the greater than additive effect on Bel W$_3$ tobacco.[357] Concentrations of either or both that may cause foliar injury are found around major metropolitan areas throughout the world and are widespread throughout rural eastern United States.[323] Macdowall and Cole reported that the two-gas combination lowered the threshold for injury of tobacco (cultivar White Gold) by sulfur dioxide, but not the threshold for ozone injury.[324] Macdowal et al. defined the threshold in terms of dose when they reported the threshold at 20 pphm-h (0.20 ppm-h). This has not

appeared true in several other reports,[357,547] nor within the results reported by Macdowall and Cole.[323] Symptoms reported, when sulfur dioxide was below the threshold for the specific plant, are similar to those reported for ozone.

Tingey et al.[547] exposed 11 species to different ratios of sulfur dioxide and ozone mixtures. There was no general trend in terms of how the ratios of pollutant concentrations influenced foliar injury; additive, greater than additive, and less than additive responses were noted (Table 11-16). They described an undersurface silvering and collapse of epidermal tissue, whereas the upper-surface injury was generally an interveinal necrotic fleck or stipple (pigment accumulation). Menser and associates[181,239,359,362] determined the response of many Nicotiana tabacum types, several Nicotiana species, and various cultivars within N. tabacum to sulfur dioxide and ozone mixtures. They found, generally, that tobacco was more sensitive to the sulfur dioxide-ozone mixtures than to the individual pollutants, but the relative sensitivity was often similar to that shown to exposure to ozone, ambient oxidant, or both. Heagle and Neely[201] compared the relative foliar injury among cotton and soybean cultivars exposed to sulfur dioxide-ozone mixtures and to the individual pollutants. The relative cultivar sensitivity to each pollutant was normally different. Jacobson and Colavito[261] found that sulfur dioxide at 0.04 ppm decreased the sensitivity of bean and increased that of vetch[331] to ozone during a 4-h exposure. Mandl et al. found that the response threshold of alfalfa to sulfur dioxide was increased by ozone at 0.07 ppm during a 4-h exposure. Dochinger et al.,[126] Costonis,[83] and Houston[249] presented evidence that ozone-sulfur dioxide mixtures change the response of white pine to the individual pollutants. Dochinger et al.[126]

Table 11-16

Summary Effects of Sulfur Dioxide and Ozone Mixtures on Foliar Injury[a]

Plant Species	Response at Concentration Ratio, SO_2:O_3, ppm[b]			
	0.50:0.05	0.10:0.10	0.25:0.10	0.50:0.10
Alfalfa	-	+	+	+
Broccoli	+	+	0	0
Cabbage	0	0	0	+
Radish	0	+	+	+
Tomato	0	-	0	0
Tobacco, Bel W 3	+	0	+	+

547

[a] Data from Tingey et al.

[b] + = greater than additive, 0 = additive, - = less than additive.

and Houston[249] found that the mixture increased the amount of injury, whereas Costonis[83] reported less injury from the mixture than from sulfur dioxide alone. Both Costonis and Houston reported effects from sulfur dioxide and its mixture with ozone at concentrations of both gases well below those of other reports. It is possible that they used ultrasensitive clonal materials. Whatever the reason, this work needs verification. Applegate and Durrant[13] reported injury to peanut at sulfur dioxide-ozone concentrations and ozone concentrations well below those reported for other plants. Their work also requires substantiation. In the latter two cases, the concentrations reported are close to the detection limits of the monitors used.

Tingey et al.[545] found additive inhibition of top growth of radish and less than additive inhibition of root growth after exposure to sulfur dioxide-ozone mixtures. Tingey and Reinert[538] and Tingey et al.[548] exposed soybean, tobacco, and alfalfa to mixtures of sulfur dioxide and ozone and reported greater than additive inhibition of root growth of soybean, additive inhibition for tobacco, and less than additive inhibition for alfalfa. Heagle et al.[205] reported greater than additive effect on growth and yield in soybean, grown under field conditions, from a mixture of these gases, but the differences between the mixture and the ozone treatments were not significant. Heagle and Trent[203] reported a less than additive effect on yield of peanut from a mixture of the two pollutants.

Weber[581] reported reductions in plant growth and nematode populations from mixtures of ozone and sulfur dioxide, but these changes were similar to those caused by ozone alone.

Other combinations of pollutants with ozone, PAN, or both may be important, but have received little study. Matsushima[349] reported additive foliar effects on pinto bean and tomato from a mixture of sulfur dioxide and PAN and a less than additive effect on tomato from mixtures of ozone and nitrogen dioxide. Fujiwara[174] reported a greater than additive effect on pea from a mixture of ozone and nitrogen dioxide. Kress[290] and Kohut[287] studied the response of hybrid poplar to ozone-PAN mixtures. Kress used sequential exposures and found a greater than additive effect after most exposures; after others, he reported mixed responses. Kohut used simultaneous exposure and found all three responses in three replicates of a study. The reasons for these variations are unclear.

Fujiwara[174] and Reinert et al.[447] have recently reviewed the subject of pollutant interaction. Fujiwara gave a straightforward reporting of research that is fairly comprehensive, whereas Reinert et al. interpreted and analyzed results. Fujiwara also included some of his data on peas and spinach. His graph (Figure 11-3) showing the greater than additive response of pea to the mixtures of ozone and sulfur dioxide is of interest, because of the linear responses of the two ozone concentrations across sulfur dioxide concentration. Reinert et al.[447] developed some useful tabular material, some of which is shown in Tables 11-17 and 11-18.

Studies of pollutant interactions are preliminary. We are still not able to define adequately the total potential impact of pollutant combinations on the production of quality food, feed, and fiber. We do know that plant species respond differently to pollutant combinations and that responses can be additive, greater than additive, or less than additive. We still do not understand variations in species or cultivar responses or the responses of plants grown or exposed under a variety of environmental stresses.

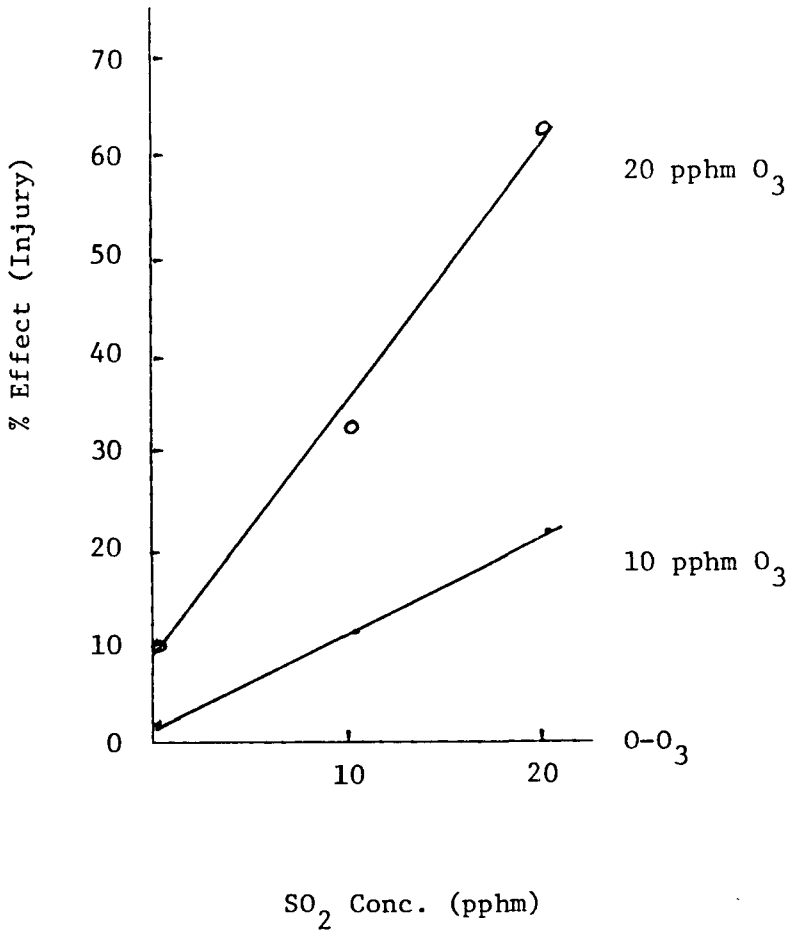


Figure 11-3. The effects of sulfur dioxide and ozone on percentage foliar injury to garden pea. (Reprinted with permission from Fujiwara[174])

Table 11-17

Foliar Response of Selected Plants
to Sulfur Dioxide and Ozone Mixtures

Plant Species	Concentration Ratio, SO_2:O_3, ppm	Exposure Duration, h	Foliar Injury, %	Response to Mixture	Reference
Bean, garden	1.70:0.19	0.5	24	+	349
Bean, lima	0.25:0.05	4	0	0	547
Broccoli	0.50:0.05	4	17	+	547
Cabbage	1.00:0.10	4	28	0	547
Tomato	0.10:0.10	4	10	-	547
Radish	0.50:0.10	4	50	+	547
Alfalfa	0.50:0.10	4	60	+	547
Eastern white pine	0.025:0.05	6	26	+	249
Cotton (6 cultivars)	1.00:0.30	6	10-24	0	201
Tobacco, cultivar Bel W 3	0.25:0.03	4	41	+	357
Tobacco, cultivar Bel W 3	0.50:0.10	4	88	+	547
Tobacco, Md (6 cultivars)	0.50:0.10	2	20	+	362

447

Data from Reinert et al.

+ = greater than additive, 0 = additive, - = less than additive.

Table 11-18

Growth Response of Selected Plants [a] to Sulfur Dioxide and Ozone Mixtures

Plant Species	Concentration Ratio, SO_2:O_3, ppm	Exposure Duration, h	Plant Response, % reduction [b] from control	Response to Mixture [c]	Reference
Radish	0.05:0.05	8/day, 5 days/wk, 5 wk	10 TDW 55 RDW	0 -	545
Radish	0.45:0.45	4	16 TDW 70 RDW	0 0	447
Alfalfa	0.05:0.05	8/day, 5 days/wk, 12 wk	18 TDW 24 RDW	- -	447
Soybean	0.05:0.05	7/day, 5 days/wk, 3 wk	24 RDW	+	548
Soybean	0.10:0.10	6/day, 5 days/wk	52 TDW 63 seed wt	0 0	205
Tobacco	0.05:0.05	7/day, 5 days/wk, 4 wk	32 TDW 49 RDW	0 0	447

[a] Data from Reinert et al. 447

[b] TDW = top dry wt, RDW = root dry wt.

[c] + = greater than additive, 0 = additive, - = less than additive.

Pollutant-pathogen interactions. An important factor in the response of vegetation to oxidants (primarily ozone) is the presence of biotic pathogens. Such responses have been studied from several perspectives since Yarwood and Middleton[596] accidentally found that rust-infected bean leaves were less sensitive to photochemical oxidants (probably PAN). Several investigators have looked at the protection from ozone injury afforded to plants with active infections; others have noted that ozone injury increases the sensitivity of plants to infection; some have studied the effects of ozone on pathogens; and several have found no interacting effects.

Yarwood and Middleton[596] reported the first protective effects in 1954. Little more was done until about 1968. Protection of plants from ozone has been shown in several instances: rust infection of wheat;[200] *Botrytis cinerea* on broad bean;[328] *Pseudomonas phaseolicola* on pinto bean;[279] mosaic virus on tobacco;[46,385] three tobacco viruses;[443] and mosaic virus on bean.[110,111] In the latter two cases, protection was reported without visible symptoms of the virus. This may be a general phenonemon, inasmuch as some protection was reported with very mild symptoms by Brennan and Leone.[46] This protective action has generally been ascribed to the production of a diffusible substance by the invading pathogen. This might be due to an alteration in plant metabolism caused by the viral infection.

Several investigators have reported that ozone injury increases the infectivity of some weak pathogens. This was originally investigated when a high incidence of *B. cinerea* was reported on potato that appeared to have severe ozone fleck.[346] The authors later reproduced the symptoms and disease incidence in greenhouse exposures. *B. cinerea* also invades ozone-injured geranium leaf tissue more rapidly.[336] It was suggested that the ozone

injured geranium leaf tissue more rapidly.[336] It was suggested that the ozone lesions serve as infection sites for the weak pathogens.

Plant pathologists have used the possibility of pathogen-pollutant interactions to study the direct effects of ozone on the pathogen while it is active on plant tissue. This is the best way to study the effects of ozone and is the only way to study these effects on obligate parasites. The results have included reduced growth of crown rust uredia on oat[195] and bean,[452] reduced hyphal growth and number of urediospores of wheat stem rust,[199] reduced infectivity of barley by powdery mildew conidia,[202] and a small reduction in symptoms caused by Fusarium oxysporium on cabbage.[344] In general, it is felt that the results are due to changes in the host physiology, and not to direct effects on the pathogen. This may not be true for reduced infectivity of fungal spores.

There are several reports of the use of ozone with biotic pathogens, and the responses noted were independent of each other. These reports include Lophodermium pinastri infection of white pine,[86] Fusarium infection of cabbage,[344] Botrytis infection of poinsettia bracts,[339] rust on bean,[452] and brown root rot of tomato.[335] These results could reflect the conditions used.

Heagle[196] has developed an excellent review of this entire subject. He has also covered the direct effects of pollutants on pathogenic organisms. We have used the portion of this tabular material that covers the effects of ozone on plant diseases (Table 11-19).

Other factors. Stark et al.[494] and Miller et al.[378] reported that oxidant (ozone) injury to ponderosa pine predisposed the trees to later invasion by pine bark beetles. The beetles increase the rate of decline and

Table 11-19

Effects of Ozone on Plant Diseases[a]

Disease Affected	Effects	Ozone Concentration and Time	Reference
Botrytis on gladiolus and chrysanthemum petals	Fewer infections by conidia	Not measured	329, 330
Botrytis on geranium petals	Decreased pathogenesis	0.35 ppm, 4 h	337
Oat crown rust	Smaller pustules	0.10 ppm, 6 h/day, 10 days	195
Wheat stem rust	Fewer infections by urediospores	0.06 ppm, 6 h	199
Wheat stem rust	Decreased hyphal growth	0.06 ppm, 6 h/day, 3 days	199
Wheat stem rust	Decreased urediospore production	0.06 ppm, 6 h/day, 17 days	199
Barley powdery mildew	Fewer infections by conidia	0.10 ppm, 24 h after inoculation	202
Barley powdery mildew	Fewer infections by conidia	0.25 ppm, 8th through 12th h after inoculation	474
Botrytis on potato leaves	Increased incidence and pathogenesis	Ambient	346
Botrytis on broad bean leaves	More infections and pathogenesis	0.15 ppm, 8 h	327
Botrytis on potato leaves	More infections and pathogenesis	0.15 - 0.25 ppm, 6 - 8 h	346
Botrytis on Geranium leaves	More infections and pathogenesis	0.07 - 0.10 ppm, 10 h/day, 15 days	336
Tobacco mosaic virus	More infections on pinto bean	0.30 ppm, 6 h	46

[a]Under ambient conditions, pollutants often exist in mixtures. Therefore, some of the effects observed in the field (ambient) may have been caused by interactions of more than one pollutant. Adapted from Heagle.[196] All work done in United States.

may be the final cause of tree mortality [77] (see Chapter 12). It is possible that oxidant stress in other parts of the country contributes to insect infestation in forest areas. Weber [581] has shown that ozone and mixtures of ozone with sulfur dioxide (0.25 ppm, 4 h/day) can decrease the population of four nematodes associated with soybean. These types of interactions may be significant in areas of the country with significant oxidant pollution problems.

Heagle and Heck [198] found that Bel W 3 tobacco was predisposed to later oxidant injury by exposure to ambient pollutants. Macdowall [320] reported the same when the preexposure was to low oxidant and the later exposure was also low. Antagonism was noted when both doses were high.

Sensitivity of plants to ozone and PAN is conditioned by leaf maturity (Table 11-20). This has received its best documentation in the work by Bobrov. [36] Middleton et al. [368] found that pinto bean primary leaves were most sensitive at the age of 14 - 34 days when exposed to an ozone-hexene mixture. Under ambient oxidant conditions, leaves of White Gold tobacco did not fleck until a week or so after the logarithmic growth phase. [320] Ting and Dugger [529] found that cotton leaves were most sensitive at about 70% expansion and that sensitivity was rapidly lost. Stomatal resistance was low, even if plants were not resistant, all other things being equal. Ting and Mukerji [531] associated this with reduced amino acid and carbohydrate pools. There was a twofold increase in amino acids 24 h after a 1-h exposure to ozone at 0.80 ppm; it was a transient increase. This suggests that low pool compounds could reduce the speed of repair mechanisms. Tingey et al. [542] reported that the first trifoliate leaf of soybean was most sensitive during the latter stages of leaf expansion. However, their metabolite studies suggested that metabolic pools did not directly affect foliar sensitivity.

Table 11-20

Response of Most Sensitive Plant Tissue to Ozone Exposures

Plant Species	Ozone Concentration, ppm, h	Leaf Type	Plant Age, wk	Effect	Reference
Pine, Virginia	0.25, 4	Cotyledon Primary Secondary	3-5 4-6 3-14	50% injury 25% injury 30% injury	114
Pine, Virginia	0.25, 2	Primary	2	58% seedlings affected	27
Pine, shortleaf	0.25, 2	Primary	2	45% seedlings affected	
Pine, loblolly	0.25, 2	Primary	2	45% seedlings affected	
Pine, slash	0.25, 2	Primary	2	34% seedlings affected	
Maple, red	0.75, 7h/day, 3 days	True	(90% expanded)	--	564
Cotton, cultivar Acala	0.70, 1	1st true	(2/3 expanded)	50% injury	529
Bean, cultivar Pinto	0 -hexene 3	Primary	2-5	--	368
Soybean	0.50, 2	1st trif.	(3/4 expanded)	45% injury	542

Townsend and Dochinger[564] did definitive work on red maple seedlings exposed to ozone at 0.75 ppm, 7 h/day over 3 days. They reported that leaves of seedlings about 90% expanded were most sensitive, but that young leaves were tolerant.

Generally, studies have shown that plants are most sensitive to ozone at a physiologic age associated with nearly expanded leaves. Sensitivity is associated with functional stomata, intercellular spaces, and rate of cutin formation on cell walls.[36] This is not true in the case of PAN, in which leaves of a lower physiologic age, just before maximal leaf expansion, are most sensitive. Plants generally are more sensitive to oxidant pollutants during rapid growth stages and lose sensitivity as leaves mature. When oxidant episodes occur throughout the growing season, the older leaves, weakened during their stage of maximal physiologic growth, show early senescence.

Hanson et al.[190] reported an increased tolerance in petunia cultivars as they approach the flowering stage. This was true for both sensitive and tolerant cultivars, with the latter more strongly influenced. They suggested that bud development produced a diffusible substance that moved down the plant and acted as a protectant.

Adedipe et al.[6] found that Bel W[3] tobacco leaves were more sensitive to ozone when attached, rather than detached or used as leaf disks. Effects were seen as visual injury and change in chlorophyll content.

Davis and Wood[107, 114] found that ages of Virginia pine needles influenced their response to a 4-h exposure to ozone at 0.25 ppm. Generally, cotyledons were more sensitive than primary needles, which were more sensitive than secondary needles. Secondary needles of seedlings were

about as sensitive as those of 3-year-old trees. This suggests that seedlings may be good test plants for determining the sensitivity of mature trees. The cotyledons and secondary needles became resistant after 16 and 18 weeks, respectively, whereas the primary needles remained sensitive beyond 18 weeks. Berry[27] reported that Virginia, shortleaf, loblolly, and slash pines at 2 - 6 weeks from seed were most sensitive to ozone at 0.25 ppm for 2 h. Most species had peak sensitivities at about 2 weeks for the primary needles. The species were listed in order of sensitivity. These results may not be contradictory, inasmuch as the exposures and growth conditions varied greatly between the two reports.

Ozone is known to reduce nodulation in soybean[450, 536] and in ladino clover.[286] The reduction in fixed nitrogen was related to a reduced nodule number, and not to nodule size. Blum[33] found no direct effect of ozone on Rhizobium or on nodule formation. He attributed the reduction in nodule number to the reduction in available energy in the root tissue. This reduction in nitrogen fixation could affect total biomass and agricultural production, especially in areas of high oxidant pollution and low soil nitrogen. Kochhar[286] also reported an inhibition of plant growth and nodulation of Trifolium repens (clover) when the plants were treated with root exudates from fescue grass exposed to ozone. If these factors are widespread, they could change the competitive ability of plant species, with a resulting change in plant diversity and a possible decrease in agricultural productivity.

Although there has been considerable interest in understanding how various factors, including air pollutants, affect the response of plants to pesticides, especially herbicides, very little has been done with air pollutants. Hodgson and associates[240-242] first showed an effect of ozone

on the metabolism of herbicides. They found that ozone inhibited the dealkylation of atrazine in corn and altered the pathway of diphenamid metabolism in tomato. The changes could be beneficial, if oxidants increase pesticide degradation, or harmful, if oxidants stop biologic breakdown at a toxic intermidiate. Carney et al.[66] reported that the herbicide pebulate in combination with ozone gave a greater than additive response on White Gold tobacco and that chloramben did the same with Delhi 34 tobacco. They reported a less than additive response of both tobacco cultivars to the combination of benefin and ozone. These and other herbicides acted independently of ozone exposure on tomato and white bean.[414] Ordin et al. found that Avena coleoptile growth was less inhibited by PAN when 2, 4-D was used in amounts giving optimal growth. These interactions with herbicides need additional investigation, to determine whether the responses noted are of general importance. Research needs to be directed at possible interactions between atmospheric pesticides (vapors or fine particles) and oxidant air pollutants.

Another interaction has recently been reported between cadmium applied to soil and ozone exposure of cress.[100] If cadmium potentiates the ozone response of cress, maybe other heavy metals respond in a similar fashion.

Discussion. A complete understanding of the many factors that affect the response of vegetation to oxidant pollutants is probably impossible. An understanding of the individual factors is possible, however, and much is already known; but the interactions between some of these many factors are unclear. It is possible that, as the mechanism of response becomes better understood, we can develop an expectation as to how various factors will interact. However, a well-trained and knowledgeable investigator can

develop a subjective estimate of response that is repeatable. Inherent genetic resistance is probably the most important factor that affects the response of a plant to an oxidant dose. However, the factors discussed here will influence the severity of response of sensitive genotypes. Even normally resistant genotypes can be injured by appropriate combinations of other factors.

Genetic or other factors that induce stomatal closure will reduce plant sensitivity to oxidant pollutants. Generally, the sensitivity of plants at the time of exposure is controlled primarily by factors that affect the stomatal aperture. The internal resistance to gas flow may also influence leaf sensitivity. Factors that affect sensitivity during growth usually cause physiologic changes in the plant that tend to make it more resistant to the added stress of oxidant. Many of these stresses may alter membrane physiology and make the membranes either more or less sensitive to oxidant stress.

Dose-Response Relationships

The development of criteria for setting air quality standards requires a sufficient data base relating a given dose (concentration of pollutant x duration of exposure) of oxidant (e.g., ozone or PAN) to some meaningful effect on plants. An understanding of dose response is also important for a basic understanding of the mechanism of oxidant effects on plants. Heck and Brandt [211] have suggested that an ideal criterion would be a set of standard equations that would relate response to concentration and duration of exposure and that would reflect the effects of all other factors that control the response of the plant. If these equations were developed, they would be different for acute and chronic exposures and perhaps specific for individual species or cultivars. Such a depth of coverage would have little practical value for

overall understanding or for ambient air quality standards. However, some average equation involving groupings of plants expressed with confidence limits might permit a more reasonable interpretation of dose-response functions under average ambient conditions. Discussions of the relationship of time, concentration, and response are found in several publications.[211, 318, 401]

Data regarding the chronic effects of oxidants (including ozone and PAN) have not been gathered on a scale wide enough to permit the development of usable equations in regard to concentration and duration of exposure. Much of the information available was discussed and tabulated earlier in this chapter. The data suggest that chronic and acute-chronic effects with resulting yield and biomass reductions can occur when average concentrations of oxidant (ozone) are between 0.05 and 0.10 ppm for 2-6 h/day over a number of days during the growing season. This type of statement is not yet possible for PAN, but in the Los Angeles basin it is always a factor in ambient-oxidant studies.

Even that kind of information is not available for forest species. Other than chronic injury to white pine (associated with ozone, sulfur dioxide, and their mixtures), no clearly defined examples of chronic injury from ozone have been reported for eastern forests, and no information is available on PAN. It is of interest that both Virginia and jack pine appear more sensitive than white pine to acute ozone exposures,[25, 112] but chronic symptoms have not been observed in either species. The relationship between oxidant dose and injury in the San Bernardino Mountains area[507] suggests that ponderosa pine is moderately to severely injured in areas that receive oxidant at above 0.08 ppm for 12-13 h each day (Chapter 12). Ponderosa pine seems to be the most sensitive western pine, but in some areas Jeffrey pine is about as sensitive.

White fir, incense cedar, and sugar pine all appear more tolerant, even to the high oxidant concentrations in the San Bernardino Mountains.[373] PAN may play some role in the chronic responses noted in the western forest species, particularly by broadleaf deciduous trees and some shrubs.

The information available on exposures to PAN and its homologues is not sufficient to develop dose-response curves. Taylor[506] reported comparative results from exposures to PAN, PPN, PBN, and P$_{iso}$BN. He found increasing sensitivity in the first three homologues. Drummond[128] found a nonlinear response of petunia to PAN that was similar to those reported for ozone. Representative information for PAN is presented in Table 11-21. Davis[108] was unable to injure cotyledons or primary needles of ponderosa pine with PAN concentrations of 0.08, 0.20, or 0.40 ppm during 8-h exposures. A species-sensitivity table for PAN was developed in the Atlas.[510]

The information on acute exposures of forest species to ozone is limited.[25, 107, 112, 125, 227, 373, 589, 594] These results of these reports suggest that many eastern deciduous species are sensitive to exposures to ozone at 0.20-0.30 ppm for 2 - 4 h. Generally, the eastern conifers are somewhat more sensitive, and the western conifers, somewhat less sensitive. These results are included in the summary data presented in this section. Treshow and Stewart[568] developed an extensive list (70 plants) from two plant communities, but it is impossible to determine percentage effect from their data. They found some injury to several species at 0.15 ppm for a 2-h exposure. This list of plants should interest investigators concerned with plant communities.

Table 11-21

Effects of Time and Concentration on
Foliar Response of Selected Plants to PAN

Plant Species	PAN Concentration, ppm	Time, h	Plant Response	Ref
Controlled Exposures:				
Petunia	0.10	5	Severe injury	496
Petunia	0.12	1	11% injury	162
Petunia, cultivar Rosy Morn	0.14	1	33% injury	506
Petunia, cultivar White Cascade	0.05	2	23% injury	128
	0.10	1	19% injury	
	0.20	0.5	15% injury	
Bean, cultivar Pinto	0.02	8	44% injury	506
	0.04	4	90% injury	
	0.14	1	55% injury	
Bean, cultivar Pinto	0.10	5	Moderate injury	496
Bean, cultivar Sanilac	0.12	2	64% injury	495
Acer, *Fraxinus*, *Gleditsia*, and *Quercus* spp.	0.20-0.30	8	Variable symptoms; sensitivity related to tissue age	127
Ambient Exposures:				
Petunia, tomato, swiss chard, others	0.025-0.03	Several	Typical symptoms	560
Petunia, tomato	ca. 0.014	4	Acute injury	506
Petunia, tomato, Romaine lettuce, others	0.015-0.02	4	Acute injury	510
Tomato, cultivar Pearson	0.10	5	Severe injury	498

This section deals with published data related to short-term exposures of sensitive plants to ozone and the resulting responses. In most cases, the measure of response is a subjective estimate of visible injury. However, various growth measures have been reported in some research, and correlations between injury and growth measures are often possible (Table 11-3). These acute ozone effects have received sufficient study to permit the construction of preliminary models to relate time, ozone concentration, and plant response for a number of plant species.

A graphic expression was developed for pinto bean and Bel W[3] tobacco exposure to ozone by Heck and Dunning.[217] Later work with a number of plants permitted the development of a simplistic model derived as an empirical relationship between ozone concentration, time, and response;[214] this gave a reasonable interpretation of acute response up through a single 8-h exposure. It also permitted the development of a reasonable acute threshold concentration for a number of species. The equation was a variant of the O'Gara equation for sulfur dioxide and is shown as:

$$C = A_0 + A_1 I + A_2 / T,$$

where C is concentration of ozone; I is the response measure, in percentage injury or percentage reduction from control; T is time, in hours; and the constants A_0, A_1, and A_2 relate to inherent and external factors affecting sensitivity. Heck and Brandt[211] and Linzon et al.[318] critically reviewed these dose-response relationships. They summarized much of the available acute-response data and analyzed the data on the basis of the O'Gara equation. Heck and Brandt[211] reported the 95% confidence curves for 5%

and 33% response; Linzon et al.[318] reported the curve for 5% response, along with data points. They constructed a threshold curve from the minimal data points shown. These reviews attempted to develop an average response across much of the useful information on acute plant response.

Larsen and Heck[297] analyzed data on the foliar response of 14 plant species to ozone (from Heck and Tingey[214] and Heck et al.[217]). They depicted the data using a mathematical model with two characteristics: a constant percentage of leaf surface is injured by an air pollutant concentration that is inversely proportional to exposure duration raised to an exponent (Figure 11-4); and, for a given exposure duration, the percentage leaf injury as a function of pollutant concentration fits a log-normal frequency distribution (Figure 11-5). The complete leaf injury equation combines the equations shown in Figures 11-4 and 11-5. This equation expresses pollutant concentration as a function of the other variables:

$$c = m_{g\,hr}\, s_g^{Z}\, t^{p},$$

where c is concentration in parts per million, $m_{g\,hr}$ is geometric mean concentration for 1-h exposure, s_g is standard geometric deviation, Z is number of standard deviations from the median (injury), t is time in hours, and p is slope of injury line on logarithmic paper. From the data for pinto bean in Figures 11-4 and 11-5:

$$c = (0.31)1.44^{Z}\, t^{-0.57}.$$

Thus, c = 0.10 ppm to give 10% injury in a 3-h exposure. The summary table of Larsen and Heck for the exposure of 14 plant species (two cultivars of corn) appears as Table 11-22. From this analysis, they suggested that averaging times of 1, 3, and 8 h should be used in the development of oxidant standards for the protection of vegetation.

For the present report, the literature was critically reviewed for information on the three measures--duration of exposure, ozone concentration, and plant response. From this review, 74 references were found to have usable data. Several criteria were established before data were used: the monitoring technique and method of calibration should be defined (40% of the references used a calibrated Mast instrument, 50% used the Mast uncalibrated or the calibration procedure was impossible to determine, 4% used a 20% buffered potassium iodide wet chemistry, 3% were unknown but other procedures suggested that the data were reliable, one report used an ultraviolet method, and one used an automated iodometric technique); the plant response must be subject to the development of a percentage evaluation in comparison with the control plant (about 93% of the responses were foliar injury--for these we often made a subjective evaluation of scales used, on the basis of injury description given--other responses included a reduction in chlorophyll, yield, leaf area, root dry weight, or top dry weight and an increase in respiration; and the general experimental procedures used needed to conform to those generally reported around the country. The data used came from a number of laboratories where growth and exposure conditions were highly variable and plants were exposed at different ages, at various times of year, and under various soil conditions. The investigators always attempted to grow and expose plants under sensitive conditions. We have assumed that these variations have given us some average over environmental conditions. The above

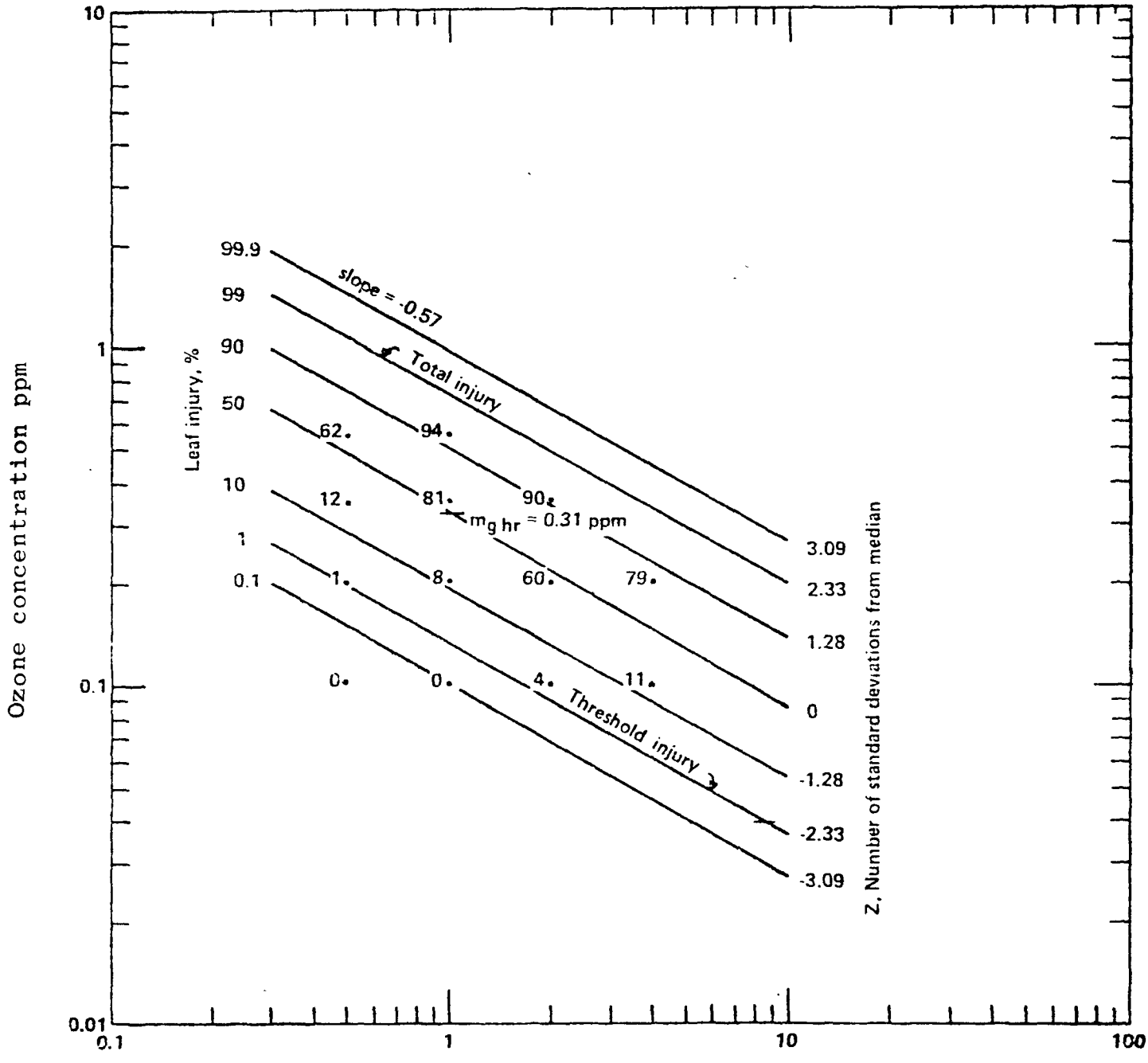


Figure 11-4. Percentage leaf injury in pinto bean plants exposed to various ozone concentrations for various durations--concentration versus exposure duration. $^{m}g=^{m}g\ hr^{tp}$, where ^{m}g=geometric mean concentration for a particular exposure duration, $^{m}g\ hr=^{m}g$ for 1 h, t = time in hours, and p=slope of injury line on logarithmic paper. (Reprinted with permission from Larsen and Heck.[297])

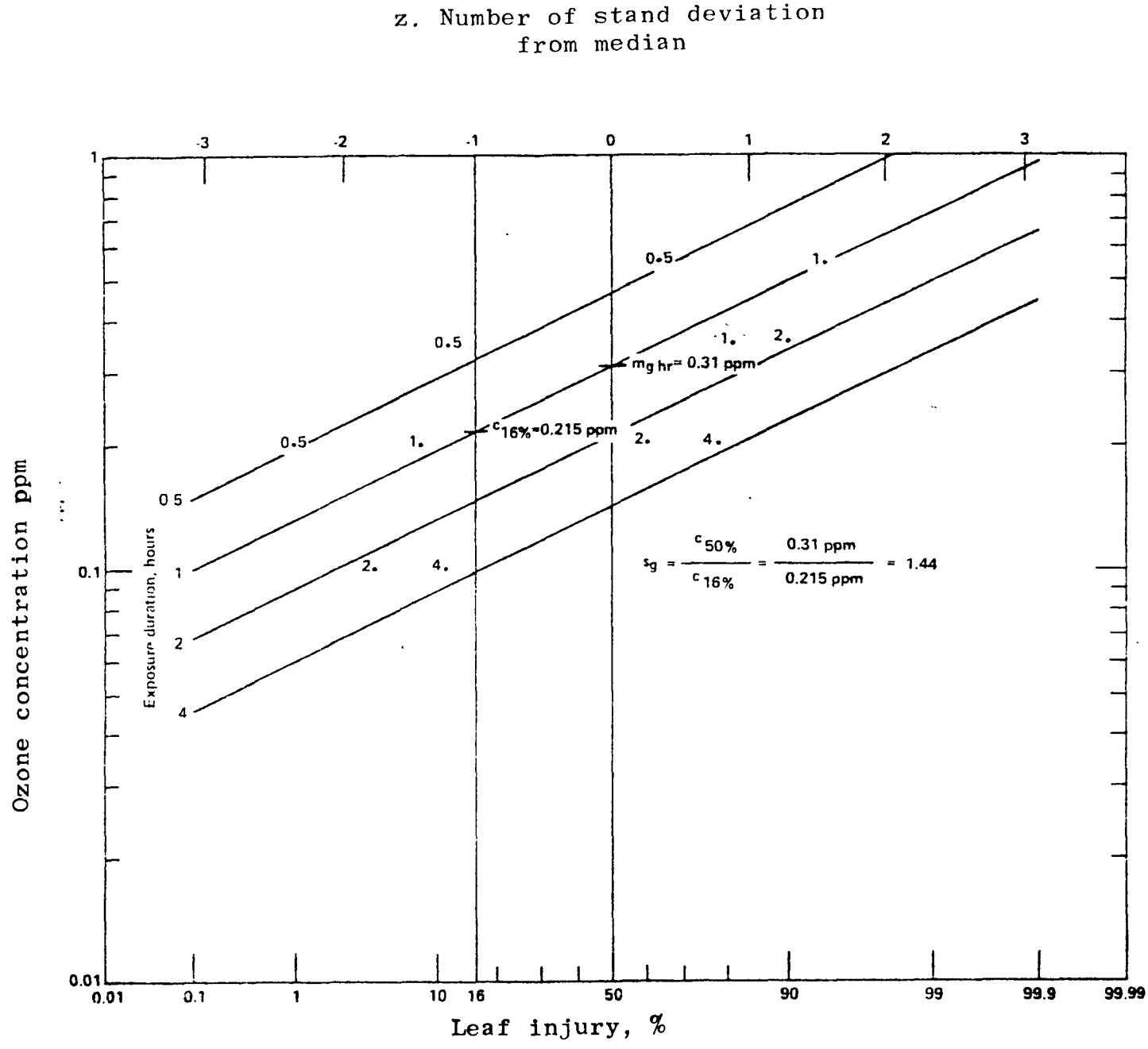


Figure 11-5. Percentage leaf injury in pinto bean exposed to various ozone concentrations for various durations--concentration versus percentage injury. $c=m_g s_g^Z$, where c=concentration in ppm, m_g=geometric mean concentration for a particular exposure duration, s_g=standard geometric deviation, and Z=number of standard deviations that the percentage of leaf injury is from the median. (Z for 10% injury is -1.28.) (Reprinted with permission from Larsen and Heck.[297])

Table 11-22

Calculated Injury Parameters for Plants Exposed to Ozone

Pollutant and plant	Calculated injury threshold* (ppm) for exposure of			Leaf injury equation** parameters			Multiple correl. coeff.	Injury conc. ratio
	1 hr	3 hr	8 hr	$m_{g\ hr}$	s_g	p		Median Threshold
Ozone								
a. Bean, Pinto	0.10	0.05	0.03	0.31	1.44	-0.57	0.98	3.1
b. Tomato, Roma	0.06	0.04	0.03	0.24	1.53	-0.35	0.98	3.7
c. Clover, Pennscott Red	0.13	0.08	0.05	0.65	1.68	-0.43	0.89	4.9
d. Tobacco, Bel W-3	0.08	0.04	0.02	0.34	1.60	-0.68	0.93	4.3
e. Spinach, Northland	0.17	0.09	0.05	0.72	1.60	-0.55	0.89	4.2
f. Chrysanthemum, Oregon	0.21	0.16	0.12	1.79	2.00	-0.27	0.76	8.6
g. Begonia, Thous. Wonders	0.11	0.08	0.06	0.76	1.85	-0.31	0.90	6.7
h. Corn, Pioneer 509	0.11	0.06	0.03	0.80	1.91	-0.57	0.94	7.4
i. Corn, Golden Cross	0.08	0.05	0.03	0.83	2.11	-0.44	0.99	10.0
j. Bromegrass, Sac Smooth	0.10	0.07	0.05	0.64	1.80	-0.38	0.95	6.2
k. Oats, Clintland 64	0.05	0.03	0.02	0.47	2.02	-0.41	0.91	8.8
l. Radish, Cherry Belle	0.05	0.04	0.03	0.29	1.72	-0.32	0.93	5.4
m. Periwinkle, Bright Eyes	0.34	0.22	0.15	1.37	1.57	-0.40	0.94	4.0
n. Wheat, Wells	0.30	0.18	0.11	0.83	1.39	-0.48	0.94	2.8
o. Squash, Summer	0.11	0.11	0.10	0.70	1.83	-0.01	0.96	6.5

* The injury threshold is arbitrarily defined here as 1 percent leaf injury in the median plant of an exposed set

** $c = m_{g\ hr} s_g^{Z} t^{p}$.

data (1,135 data points) were divided into three susceptibility groupings, on the basis of information from Table VIII in reference 211 and Table 6-6 in reference 401. These tables were modified, on the basis of the analysis of the data used (Table 11-23). The three susceptibility groups were: sensitive (471 data points), intermediate (373 data points), and resistant (291 data points). The plants (and appropriate references) in each susceptibility group are shown in Table 11-24. The underlined references (in the table) were not used in compiling the data for the generation of Figure 11-6 and Table 11-24a.

The data developed for each susceptibility group were then analyzed with the equation of Heck and Tingey[214] (Figure 11-6 and Table 11-24a). This is not to say that the data generated could not be used to develop a different or more complex model. However, this model presents a better interpretation of data than other simple models that have been used. It should be emphasized that the data presented are pertinent only to pollutant concentrations that produce an acute response in 8 h or less (two data points in the sensitive group were for 12 h). None of the information should be extrapolated beyond this period. The data from exposure of the plants in Table 11-24 (except for underlined references) were used in the model. Several rather extensive lists of species and cultivar sensitivities to oxidants, ozone, and PAN are found in a number of references.[67,235,236,269,401,475,510,515,568]

Table 11-23

Ozone Concentrations for Short-Term Exposures That Produce [a] 5% or 20% Injury to Vegetation Grown under Sensitive Conditions

Exposure Time, h	Concentrations That May Produce 5% (or 20%) Injury, ppm		
	Sensitive Plants	Intermediate Plants	Resistant Plants
0.5	0.35-0.50 (0.45-0.60)	0.55-0.70 (0.65-0.85)	≥ 0.70 (0.85)
1.0	0.15-0.25 (0.20-0.35)	0.25-0.40 (0.35-0.55)	≥ 0.40 (0.55)
2.0	0.09-0.15 (0.12-0.25)	0.15-0.25 (0.25-0.35)	≥ 0.30 (0.40)
4.0	0.04-0.09 (0.10-0.15)	0.10-0.15 (0.15-0.30)	≥ 0.25 (0.35)
8.0	0.02-0.04 (0.06-0.12)	0.07-0.12 (0.15-0.25)	≥ 0.20 (0.30)

[a] Data developed from analysis of acute responses shown in Table 11-24a and Figure 11-6.

Table 11-24

List of Plant Species and Cultivars by[a] Susceptibility to Acute Ozone Exposures

SENSITIVE

Alfalfa[410]
- Dawson[257]
- Glacier[257]
- Mesa-Sirsa[257]
- Moapa[257]
- Vernal[257]
- Williamsburg[257]

Aspen, quaking[568]

Bean
- Pinto[89,109,136,139,147,212,216,217,368,433,435,464,492,513,561]
- Seeway[65,105]
- White[166]
- Sanilac[109, 580]

Broccoli
- Calabrese[214]

Chrysanthemum
- King's Ransom[284]
- Mango[284]
- Minn White[284]
- Mt. Snow[284]
- Red Mischief[284]
- Tranquility[284]

Clover, red
- Kenland[52]

Coleus
- Scarlet Rainbow[5]

Corn, sweet[62]

Cucumber[410]

Duckweed[159]

Grass, annual blue[44]

Grass, bent
- Astoria[44]
- Cohansey[44]
- Holfior[44]

Grass, bent
- Penncross[44]
- Seaside[44]

Grass, brome
- Sac Smooth[214]

Mustard[410]

Oats
- 329-80[214]
- C. I. 7540[49]
- Clintland 62[214]
- MO-0-205[49]
- Oneida[49]
- Pendek[214]

Petunia
- Capri[5]
- Pink Cascade[67]

Pine, jack[25]

Pine, red[25]

Pine, Virginia[112 113]

Pine, eastern white[25, 83]

Radish
- Cavalier[4,417]
- Champion[449]
- Cherry Belle[4,214,449]
- Crimson Giant[449]
- Comet[449]

Safflower
- Arizona 14154[256]
- Biggs[256]
- Jordan[256]
- Nebraska 1-1-5-1[256]
- Nebraska-4051[256]
- Nebraska-6[256]
- Nebraska-8[256]
- Pacific-2[256]
- Utah 1421-9-16[256]
- Ute[256]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by Susceptibility to Acute Ozone Exposures[a]

SENSITIVE

Soybean[410]
- Clark[382]
- Clark 63[382]
- Cutler[382]
- Dare[139,254,382]
- Haberlandt[382]
- Kent[254,382]
- Lee[382]
- Lincoln[382]
- Peking[382]
- Richland[382]
- Roanoke[382]
- S100[382]
- Semmes[382]
- Wye[382]

Spinach
- America[338]
- Dark Green Bloomsdale[338]
- Hybrid 612[338]
- Virginia Blight Res. Savoy[338]

Tobacco
- Bel B[214]
- Bel C[363]
- Bel W_3[6,11,64,139,212,214,216,217,352,354,361,363,464,484]
- Bel B, Callus[11]
- Bel W_3, Callus[11]
- Catterton[362]
- Coker 187[358]
- Coker 187-Hicks[358]
- Coker 316[358]
- Coker 319[358]
- Delcrest[358]
- Delhi 61[358]
- Havana 142[239]
- Havana 307[239]
- Havana 501[239]
- Havana 503[239]
- Hicks[358]
- Maryland, 6 cv[362]
- Maryland 10[362]

Tobacco
- Maryland 59[362]
- Maryland 64[362]
- Maryland 609[362]
- McNair 30[358]
- NC 95[358]
- Reams 64[358]
- Samsun[181]
- Speight G-3[358]
- Speight G-7[358]
- Speight G-36[358]
- Va 115[358]
- White Gold[214,358]
- Wilson[362]
- Xanthi[181]

Tobacco, (N. glutinosa)[181]

Tobacco, (N. rustica)
- Brasilia[181]

Tomato
- Heinz 1350[448]
- Marglobe[448]
- Pearson[448,497]
- Red Cherry[448]
- Roma[214]
- Roma VF[448]
- Rutgers[308,311]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by Susceptibility to Acute Ozone Exposures[a]

INTERMEDIATE

Alfalfa
- Cherokee[257]
- Iroquois[257]
- Kanza[257]
- MSA-CW3An2[257]
- MSB-CW5An2[257]
- MSHp6F-An2W2[257]
- Saranac[257]
- Team[257]
- Vernal[214]

Ash, white[589]

Bean[109]
- Astro[109]
- Bush Blue Lake[109]
- Clipper[105]
- Pinto[469]
- Tempo[109]

Bean, lima
- Thaxter[214]

Beet
- Perfected Detroit[214]

Begonia
- Linda[5]
- Thousand Wonders White[214]
- White-Tausendschon[5]

Cabbage
- All Season[214]

Chard, Swiss
- Fordhook Giant[214]

Chrysanthemum
- Baby Tears[284]
- Chris Columbus[284]
- Corsage Cushion[284]
- Crystal Pat[284]
- Gay Blade[284]
- Golden Arrow[48]
- Pancho[284]
- Penguin[284]
- Pink Chief[284]
- Sleighride[284]

Chrysanthemum
- Tinkerbell[284]
- Touchdown[284]
- Yellow Supreme[284]

Clover, red
- Chesapeake[52]
- Pennscott[52,214]

Clover, ladino[52]

Clover, white sweet[52]

Coleus
- Pastel Rainbow[5]

Corn
- Golden Cross[214]
- Pioneer 509[214]

Cucumber
- Chicago Pickling[433]
- Long Marketer[214]

Duckweed[90]

Grass, bent
- Highland[44]
- Kingstown[44]

Grass, Bermuda
- Kansas P-16[44]

Grass, brome
- Smooth Sac[214]

Grass, Kentucky blue
- Delta[44]
- Merion[44]

Grass, perennial rye
- Lamora[44]
- Manhattan[44]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by Susceptibility to Acute Ozone Exposures[a]

INTERMEDIATE

Grass, red fescue
- Highlight[44]
- Pennlawn[44]

Larch, European[112]

Lettuce
- Dark Green Boston[449]

Oats
- C. I. 7575[49]
- C. I. 7578[49]
- Clarion[49]
- Clintland 64[214]
- Garry[49]

Onion
- SW34[144]

Petunia
- Comanche[67]

Pine, Austrian[112]

Pine, jack[112]

Pine, Virginia[114]

Poinsettia
- Eckespoint[340]
- Paul Mikkelson[340]

Radish
- Calvalrondo[449]
- Cavalier[3]
- Early Scarlet Globe[449]
- French Breakfast[449]
- Icicle[449]
- Red Boy[449]

Safflower
- Frio[256]
- Nebraska-10[256]

Sorghum
- Martin[214]

Soybean
- Amsoy[546]
- Arksoy[382]
- Chippewa 64[546]
- Clark 63[546]
- CNS[382]
- Dare[139,542,546]
- Delmar[254]
- Dunfield[382]
- Hark[546]
- Hawkeye[546]
- Kent[546]
- Lee[546]
- Ogden[382]
- P. I. 157474[382]
- P. I. 181550[382]
- Scott[214,546]
- Traverse[546]
- Wayne[546]
- York[382]

Spinach
- Bounty[338]
- Northland[214]
- Winter Bloomsdale[338]

Spinach, New Zealand[338]

Squash, summer[214]

Tobacco
- 56-92B[352]
- Ast-C[352]
- Bel B[6,354,361,363]
- Bel C[354,361]
- Bel W_3[214]
- Catterton[352,361,363]
- Delhi 61[354]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by Susceptibility to Acute Ozone Exposures[a]

INTERMEDIATE

Tobacco
- H_2[484]
- McNair 12[354]
- NC-95[354]
- Samsun NN[181]
- White Gold[354]

Tomato
- Fireball[8, 280]
- Manapal[448]
- Ohio WR-7[448]
- Ohio WR-25[448]
- VF 13L[448]
- VF 145B7879[448]

Wheat
- Wells[214]

RESISTANT

Alfalfa
- Atlantic[52]
- DuPuits[52]
- Vernal[214]

Arborvitae[112]

Azalea
- Alaska[214]

Bean
- Eagle[109]
- Harvester[109]
- Provider[109]
- Stringless Black Valentine[109]
- Tender Crop[109]

Begonia
- Christmas[214]

Begonia
- Scarletta[5]

Chard, Swiss
- Fordhook Giant[214]

Chrysanthemum[214]
- Ann Ladygo[284]
- Bonnie Jean[284]
- Bright Yellow Tuneful[284]
- Cameo[48]
- Dark Yellow Tokyo[284]
- Distinctive[284]

Chrysanthemum
- Dolli-ette[284]
- Flair[284]
- Fuji Jess Williams[284]
- Fuji-Mefo[284]
- Golden Cushion[284]
- Golden Peking[284]
- Golden Yellow Princess Ann[284]
- Indian Summer[48]
- Jessamine Williams[284]
- Larry[284]
- Lipstick[284]
- Mandalay[284]
- Mermaid[284]
- Muted Sunshine[48]
- Oregon[214]
- Pink Chief[48]
- Queens Lace
- Red Dessert[48, 284]
- Redskin[48, 284]
- Resolute[48]
- Rosey Nook[284]
- Ruby Mound[48, 284]
- Silver Sheen[48]
- Spinwheel[284]
- Tinkerbell[48]
- Touchdown[48]
- Tranquility[48]
- Trident[48]
- White Grandchild[284]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by Susceptibility to Acute Ozone Exposures[a]

RESISTANT

Chrysanthemum
- Wildfire[284]
- Yellow Jess Williams[284]
- Yellow Jeanette[48]
- Yellow Moon[48, 284]

Clover
- Alsike[52]

Corn, sweet[62]

Cotton
- Acala[214]
- Acala SJ-1[529]

Cucumber
- Long Marketer[214]

Fir, balsam[112]

Fir, Douglas[112]

Fir, white[112]

Grass, Orchard
- Potomac[214]

Grass, zoysia
- Common[44]
- Meyer[44]

Hemlock, Eastern[112]

Holly, English[47]

Larch, Japanese[112]

Lettuce
- Big Boston[449]
- Butter Crunch[449]
- Dark Green Boston[214]
- Grand Rapids Forcing[449]
- Great Lakes[449]
- Imperial #456[449]
- Romaine[449]
- Simpson, Black Seeded[449]

Maple, sugar[229]

Oats
- Clintland 64[214]

Onion[214]

Periwinkle
- Bright Eyes[214]

Petunia
- Blue Danube[162]
- Blue Jeans[162]
- Blue Sea[162]
- Bonanza[5]
- Calypso[162]
- Canadian-all Double Mix[5]
- Cherry Blossom[162]
- Festival[162]
- Lilac Time[162]
- Parti Pink (Pink)[162]
- Peach Blossom[162]
- Peaches & Cream[162]
- Red Magic (Red)[162]
- Roulette[162]
- Victory[162]
- Warrior[162]

Pine, pitch[112]

Pine, red[112]

Pine, Scotch[112]

Pine, eastern white[112]

Poinsettia[214]
- Annette Hegg[68]
- Dark Red Annette Hegg[340]
- Eckespoint C-1[68]
- Mikkelwhite[340]
- White Annette Hegg[340]

Table 11-24 (Cont.)

List of Plant Species and Cultivars by[a] Susceptibility to Acute Ozone Exposures

RESISTANT

Snapdragon
- Floral Carpet[5]
- Rocket Mixture[5]

Sorghum
- Martin[214]

Soybean
- Cutler[254]
- Harosay[546]
- Hood[546]
- Pickett[546]
- Scott[214]
- York[254]

Spinach
- Viroflay[54]

Spruce, black[112]

Spruce, blue[112]

Spruce, Norway[112]

Spruce, white[112]

Sultana
- White Imp[214]

Tobacco
- Bel C[6]
- Hicks[354]

Tomato
- Fireball[280]
- Heinz 1439[448]
- VF 145B[448]

Vetch, crown
- Chemung[52]
- Penngift[52]

[a]Plants are listed by common generic names followed by species common name. Cultivars are listed under the common names. Data from the underlined references were not used in developing Figure 11-6 or Table 11-24a.

Time	Concentration, ppm		
	Sensitive	Intermediate	Resistant
0.5	0.431 ± 0.044	0.637 ± 0.043	0.772 ± 0.070
1.0	0.218 ± 0.023	0.347 ± 0.020	0.494 ± 0.028
2.0	0.111 ± 0.020	0.202 ± 0.017	0.355 ± 0.023
4.0	0.058 ± 0.022	0.129 ± 0.019	0.286 ± 0.026
8.0	0.031 ± 0.023	0.093 ± 0.021	0.251 ± 0.034

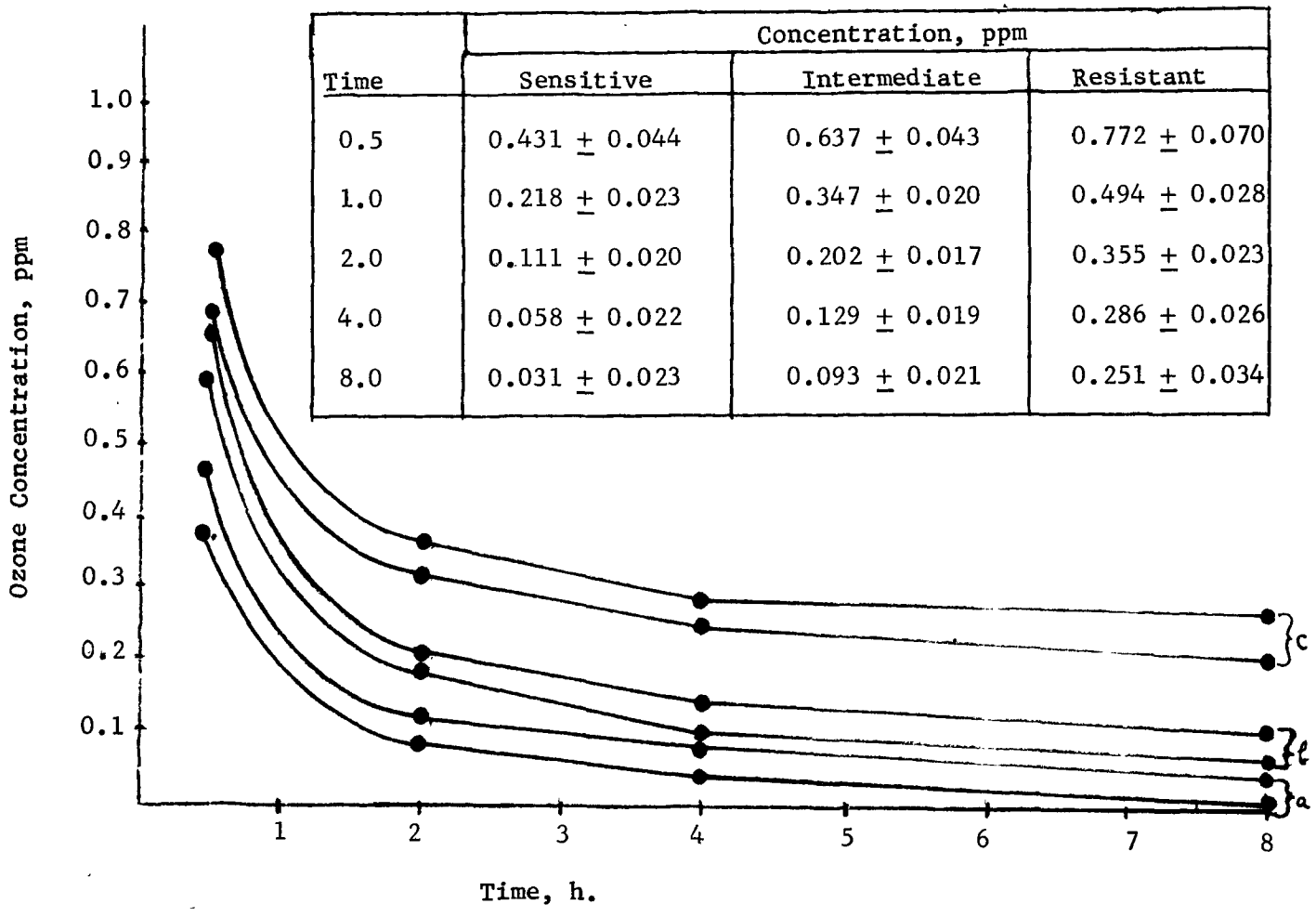


Figure 11-6. Ozone concentration vs duration of exposure required to produce a 5% response in three different plant susceptibility groupings. The curves were generated by developing 95% confidence limits around the equations for "all plants" in each susceptibility grouping from Table 11-24a. Curves: a = sensitive plants, b = intermediate plants, c = resistant plants.

Table 11-24a

Concentration, Time, Response Equations for Three Susceptibility Groups and for Selected Plants or Plant Types with Respect to Ozone[a]

Plants	$(C=A_0 + A_1 I + A_2/T)$[b]	R^2 [c]	Threshold Concentration, ppm [d]	No. Data Points	Mean Values[e] Conc., ppm	Time, h	Response, %	Dose, ppm-h
Sensitive								
All plants	C= -1.52+0.40 I+21.3/T	0.57	0.03	471	0.29	1.74	45.4	0.503
Grasses	C= -5.65+0.48 I+29.1/T	0.74	0.01	71	0.37	1.66	50.9	0.608
Legumes	C= 4.52+0.36 I+17.2/T	0.46	0.09	100	0.34	1.42	48.1	0.480
Tomato	C= -8.23+0.43 I+24.3/T	0.58	None	28	0.31	1.58	56.5	0.493
Oat	C= -4.27+0.51 I+27.3/T	0.76	0.02	30	0.37	1.66	48.2	0.611
Bean	C= -0.98+0.38 I+16.4/T	0.58	0.03	62	0.30	1.23	47.2	0.370
Tobacco	C= 2.45+0.34 I+13.7/T	0.52	0.06	197	0.23	1.98	38.9	0.448
Intermediate								
All plants	C= 2.44+0.65 I+29.0/T	0.74	0.09	373	0.37	1.67	27.0	0.625
Vegetables	C= -0.79+0.64 I+26.3/T	0.79	0.06	25	0.41	1.29	33.5	0.532
Grasses	C= 1.87+0.59 I+29.2/T	0.82	0.09	68	0.39	1.61	31.8	0.625
Legumes	C= 1.16+0.74 I+32.9/T	0.81	0.09	104	0.40	1.59	25.0	0.642
Perennial	C= 7.48+0.70 I+23.7/T	0.77	0.14	27	0.36	1.91	22.9	0.687
Clover	C= -0.99+0.71 I+26.8/T	0.95	0.06	24	0.28	2.13	23.0	0.595
Wheat	C= -0.36+0.81 I+30.2/T	0.88	0.08	15	0.47	1.25	28.9	0.588
Tobacco	C= 6.31+0.87 I+15.2/T	0.78	0.13	59	0.28	1.99	15.7	0.551
Resistant								
All plants	C= 16.89+0.95 I+27.8/T	0.51	0.25	291	0.45	1.55	10.6	0.696
Legumes	C= 8.98+1.08 I+30.4/T	0.82	0.18	36	0.38	1.89	12.2	0.722
Grasses	C= 19.06+1.17 I+26.3/T	0.55	0.28	13	0.45	1.47	6.5	0.655
Vegetables	C= 19.79+1.26 I+18.7/T	0.70	0.28	16	0.55	1.50	17.8	0.819
Woody plants	C= 23.12+0.61 I+28.8/T	0.45	0.30	46	0.39	2.50	7.8	0.985
Cucumber	C= 15.05+1.41 I+10.6/T	0.83	0.23	18	0.41	1.41	13.3	0.581
Chrysanthemum	C= 20.68+0.52 I+25.6/T	0.48	0.27	45	0.39	2.17	12.6	0.847

[a]Equations were developed from exposures limited in time (0.5 - 8 h, except for 2-12 h points in the sensitive group) and denote acute responses of the plants. Concentrations range from 0.05 to 0.99 (1.0) ppm and responses from 0 to 99 (100)% of control.

[b]C is ozone concentration in pphm; I is percent response; T is time in hours; and A_0, A_1, and A_2 are constants (partial regression coefficients) that are specific for pollutant, plant species or group of species, and environmental conditions used.

[c]Multiple correlation coefficient squared, which represents the percent variation explained by the model.

[d]For 5% response in an 8-h period.

[e]From the computer analysis.

The equations developed and the figures shown reflect the fact that concentration plays a greater role in the response of plants to ozone than does time. Although the concept has long been accepted, it is often forgotten that equal doses do not necessarily give equal responses.[217, 379] That is, a given dose applied over a short period produces a much greater plant response than an equal dose applied over a longer period. The concept of a concentration threshold actually ensures this basic dose concept. Although some disagree with the threshold concept with respect to the oxidant pollutants, the concept must have mechanistic validity, inasmuch as organisms have some inherent mechanism for detoxifying oxidants. This might not be true in the case of some individuals of a given species that might have lost the inherent protective mechanism. However, when we are dealing with a large number of species of vegetation and are attempting to develop some type of realistic oxidant dose response, we must use the species as a whole, or at least a major subdivision (cultivar) of it. In these cases, in the work that has been done, there is a rational body of data to support a threshold concept.

Plants as an Oxidant Sink

Heck and Brandt[211] discussed the effectiveness of green belts in relation to vegetation as a pollutant sink and concluded that vegetation probably acts as a major sink for air pollutants, including oxidants and ozone, over time, but has a relatively minor effect on oxidant concentrations during high-pollution episodes; is more effective in some seasons than others or with some cultural and management practices than others; and should not be considered an important contributor to short-term reductions in oxidant or ozone concentrations.

The concept of vegetation as a pollutant sink is significant to the atmospheric chemist and meteorologist who is attempting to develop air pollution material budgets. There is a need to know the ultimate sinks of air pollutants released by the activities of man. The preliminary research results available show attempts to understand the parts played by plants, soils, and soil organisms.

Turner et al.[569] reported that freshly turned soil exposed to air was a significant sink for ozone. They found that soil was about 50% as active as charcoal in removing ozone from air. Turner et al.[570] reported ozone flux within a maize field and a forest. They determined that vegetation could reduce ozone loss to soil when plants were under stress, but supported the view that vegetation and soil could be the primary sinks for ozone. Macdowall[322] found that soil absorbed twice as much ozone as mature field-grown tobacco growing on the soil. He concluded that soil is an effective sink and may play a protective role, in that it tends to keep the oxidant concentration within the canopy low. Abeles[2] suggested that most soil activity, in relation to reduced pollutant concentration, is associated with the metabolic activity of soil microorganisms. This may not be true with ozone, because it is such a reactive gas. Soils may play a more important role than has been suggested, but they are usually covered with vegetation and in general are probably less important than vegetation in acting as pollutant sinks.

Two reports[460, 527] based on rather gross measuring techniques found a close correlation between ozone uptake and transpiration. These studies indicated that stomatal control is the prime factor in controlling pollutant uptake and that cuticular sorption is negligible in relation to

stomatal absorption. These findings are generally supported by past work that indicates that stomata are the prime sites of pollutant entry into plant tissues. However, cuticular sorption may be a small but effective sink, over time. Townsend[563] used similar techniques and reported ozone sorption by nine tree species. He did not attempt to separate stomatal and cuticular sorption. White birch showed a linear uptake from 0.10 to 0.80 ppm (Figure 11-7) and only a 5% decrease in uptake rate over an 8-h exposure at 0.20 ppm. Uptake in red maple was linear to 0.60 ppm and was at only 40% of the maximal rate after an 8-h exposure to 0.20 ppm. Townsend presented values of actual uptake rates that may be of value. Several of these are given in Table 11-25. All these studies determined uptake on the basis of the time needed to deplete the ozone in a closed system. This does not permit a high reliability in the data given, but they are among the best available.

Hill[233] reported pollutant uptake values for a number of gaseous pollutants, including ozone and PAN, with alfalfa as his test organism (Table 11-25). These values were obtained with a dynamic, but closed, exposure facility. Uptake was determined by the amount of pollutant needed to maintain a constant chamber concentration over an alfalfa bed. Uptake values, expressed on the basis of leaf area, reflect the effect of the plant canopy on the exchange of gases within the canopy and do not give the maximal capability of plants for pollution sorption. They are, however, representative of the agricultural situation. The values shown are an order of magnitude below those reported by Townsend[563] and reflect the experimental conditions used, as well as the test species. Hill's use of the alfalfa canopy partially explains his lower values, but the technique used by Townsend could have resulted in inflated values.

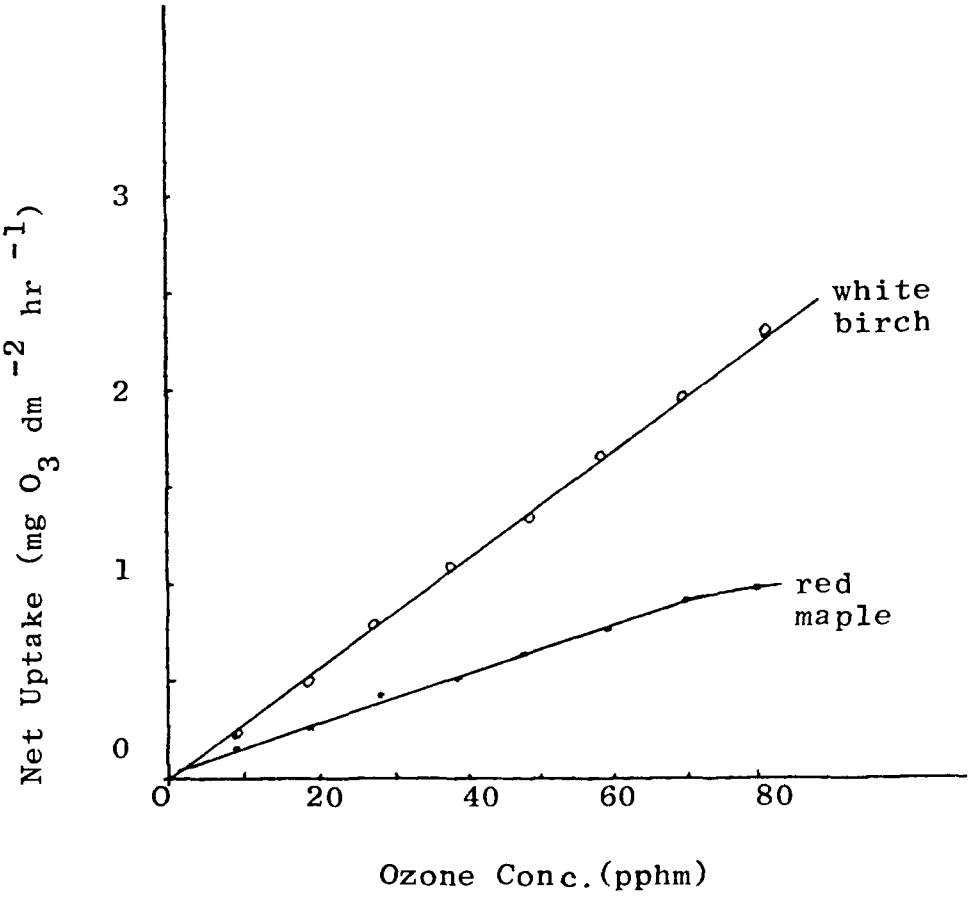


Figure 11-7. Net ozone uptake by foliage of red maple and white birch seedlings in relation to ozone concentrations. (Reprinted with permission from Townsend.[563])

Table 11-25

Rates of Oxidant Uptake by Selected Plant Species

Plant Species	Pollutant	Uptake Rate ($\mu g \cdot dm^{-2} \cdot hr^{-1} \cdot pphm^{-1}$)	Reference
White oak	O_3	32	563
White birch	O_3	27	563
Sugar maple	O_3	19	563
Sweet gum	O_3	14	563
Red maple	O_3	14	563
Alfalfa	O_3	1.1	233
Alfalfa	PAN	1.2	233

Bennett et al.[22] have presented a model for gaseous pollution sorption by plants. The model includes all the known factors that might have a significant effect on pollution sorption by plant leaves, including gas concentration (ambient air and internal leaf), gas fluxes (external and internal), resistance to flow (leaf boundary layer, stomatal, and internal), nature of leaf surfaces (stomatal presence, cutin, and surface properties), importance of gas solubility and thus solute concentration within the leaf, and ability of the plant to metabolize pollutants (decontaminate itself). They mentioned the reactivity of ozone as another factor to consider. They believe that surface sorption may be important, at least over short periods. They presented a possible mathematical representation of these factors, which they suggested is equivalent to the mathematical statement of Ohm's law. This material is well integrated in the review by Bennett and Hill.[21]

Rogers[466] used a constantly stirred tank reactor that permitted instantaneous mixing of incoming air with air already in the chamber. The system was based on a dynamic exposure design with a single-path air flow system. This system should permit the development of maximal uptake rates under given oxidant loads and a more accurate appraisal of the effects of oxidants on stomatal activity. The chamber design permits a constant turbulence within the chamber and an average air flow over leaf surfaces. Within bounds, one can vary this turbulence, while maintaining a uniform mixture of pollutants in the chamber. Preliminary work with this chamber have produced uptake rates essentially doubling those reported by Hill[233] for nitrogen dioxide. However, Rogers has not yet used the design to study other oxidants (ozone and PAN).

Oxidant uptake rates are controlled by inherent variations within plant species, the effect of the oxidant on the uptake potential of the plant, the effect of environmental stresses on the uptake potential, and meteorologic factors that affect pollutant distribution. It is important to know whether both resistant and sensitive plants act as oxidant sinks or whether only sensitive plants are major sinks. This knowledge requires some understanding of the mechanism of plant resistance to oxidants. If resistance is associated with stomatal closure due to the oxidants,[142] then resistant plants will not act as effective sinks. However, if resistance is physiologic, these plants could act as effective oxidant sinks, with little adverse effect on the plants. Neither resistant nor sensitive plants would be effective sinks under environmental stress that caused stomatal closure.

Plants probably act as major oxidant sinks over both time and distance. However, it is important to remember that plants do not respond in a predictable way over time and are not active over the greater portion of the year. The total capacity of plants as sinks with or without harm to the receptors is not known.

Plant Protection

Air pollution research in effects on vegetation has focused largely on symptom identification, dose response, and mechanisms. The purpose has been to develop criteria necessary to understand effects and to form a basis for the promulgation of ambient air quality standards. Environmentalists were not concerned with protecting plants *per se*, but in controlling emission. Thus, those interested in protection of plants received little encouragement. In addition, no economical ways were suggested for control, and research

leaders were not convinced of the importance of adding air pollution stress to breeding programs. It was the hope of most research workers that air quality standards would protect vegetation from ozone and other oxidants.

Most research workers are now convinced that pollution abatement will have little impact on overall pollution concentrations until clean energy forms are developed and in widespread use. Because phytotoxic concentrations of ozone and other oxidants are inevitable for the foreseeable future, researchers are seriously considering other means of protecting plants from injurious effects of oxidants.

For managed agricultural crops, including forest species and other woody perennials, the addition of air pollution stress into standard breeding programs has begun or is being discussed. Some workers are trying to develop protective sprays for the more sensitive species and cultivars. Some consideration is also being given to the management of cultural practices and to general land management. Ormrod and Adedipe[415] have presented an excellent review of the concepts and work to date regarding the protection of horticultural plants from atmospheric pollutants.

Breeding for resistance. Varietal studies define the genetic variability in resistance to oxidants within different species. These types of studies are necessary to select genetic lines or accessions for breeding programs. Although plant-breeders have not selected for oxidant resistance, selection probably has occurred, for two reasons: breeders normally select plants with the highest yield and least injury, regardless of cause; and many breeding programs are carried out in areas of relatively high oxidant content. In this regard, natural selection pressures should increase the tolerance of

populations of native species near urban industrial areas, although this would tend to reduce the genetic plasticity within sensitive species.

Oxidant problems are starting to be of concern to plant-breeders. Extensive varietal screening of tomato, petunia, and other plants has permitted some resistant cultivars to be recommended for use in high-oxidant areas. None of the varietal screens have involved breeding experiments in which resistant lines are developed and used in the development of resistant varieties for new introductions.

Growers in the Connecticut valley have probably selected wrapper tobacco for oxidant resistance since the 1950's. Similar selection is probably taking place in other tobacco-growing areas. Sand[472] worked with a number of wrapper selections and suggested a partial dominance of genes for fleck resistance. He found that the F_1 hybrid between a sensitive and a resistant selection was more resistant than the parental average. Povilaitis[440] used five tobacco varieties and made six crosses with six genetic populations from each cross, for 36 different populations. He used injury ratings and, unlike Sand, suggested that susceptibility may be dominant over tolerance (dominance was important in five of six crosses).

Johnson et al.[266] determined the variation in response of 16 sweet corn hybrids to ambient oxidants in California. From these observations, Cameron and Taylor[62] selected five inbreds and three of their F_1 hybrids for further field studies and four inbreds and two hybrids for use in ozone exposures. The relative effects of the polluted environments were the same. The final data suggested an inheritance for partial dominance to ozone susceptibility.

Townsend and Dochinger[564] worked with four red maple selections and found sensitive and tolerant selections that showed the same relative

susceptibility over four growth stages and four leaf developmental phases. Symptoms were similar in the four selections, but the design should have used lower ozone dosages. These results suggest a strong genetic control that will facilitate selection of ozone-tolerant seedlings for urban use.

Houston[248] and Houston and Stairs[250] did clonal repeatability analyses to determine genetic control of tolerance in white pine with an ozone-sulfur dioxide mixture and a 6-h exposure. They used needle elongation and two injury estimates in assessing effects. The repeatability estimates indicated that tolerance to the pollutant mixture is under genetic control. The nature of the inheritance of tolerance is still not understood, but field selection of tolerant or susceptible individuals is possible. Demeritt et al.[122] reported an evaluation system that used visible needle injury for determining resistance of Scotch pine to ozone. This permitted the discrimination of phenotypic differences in a quantitative way. Results suggested that a few genes were responsible for resistance in Scotch pine. A program was initiated to select for ozone resistance in loblolly pine,[582] but no definitive results are available.

As genetic understanding develops, it will be incorporated into basic breeding programs that are concerned with such characteristics as yield, growth habit, insect and disease resistance, flavor, and texture. These programs will be most effective if present ambient ozone and other oxidant concentrations do not increase.

Protectant sprays. Kendrick et al.[278] first reported protection of pinto bean foliage from sprays or dusts of four fungicides. They later

reported rather extensive studies that used a number of fungicides and antioxidants on selected test plants.[277] These tests were in general agreement with the earlier studies and suggested that the response was due to a surface deactivation of ozone and other oxidants. Jones[267] found protection of tobacco with various particulate substances, including charcoal. Freebairn and Taylor[170] found protection from use of ascorbate sprays as antioxidants. They reported partial protection to pinto bean, petunia, celery, lettuce, and grapefruit from oxidant injury in the Los Angeles basin. Several other early studies reported various degrees of chemical protection from fleck injury to tobacco.[30, 575] Taylor and Rich[502] found that an antiozonant-treated cloth protected wrapper tobacco from oxidant injury.

These early studies, along with the recognition that oxidant pollution will continue as a significant problem, encouraged a number of studies directed at finding chemical treatments that might serve a dual function with a minimum of applications. Some selected results of these studies are summarized in Table 11-26.

The fungicides, as a group, have received the most attention, and Benomyl (methyl-1-butylcarbamoyl-2-benzimidazolecarbamate) has been most widely studied, owing to its protective properties. Benomyl has been used as a foliar spray, a soil drench, and a soil amendment. Mixed results have been reported when benomyl was used as a spray. Protection was reported on tobacco,[446] pinto bean,[334, 343, 479] white bean,[99] and grape;[276] no protection was found for pinto bean,[433, 434] and cucumber.[433] It was not possible to equate the Benomyl concentrations used, but most were 20 - 4,000 ppm. Most reports on soil applications showed protection against oxidants. Soil drenches at 10-1,000 ppm gave complete to partial protection for

Table 11-26

Protection of Plants from Oxidant Injury by Application of Protective Chemicals[a]

Plant Species	Pollutant Protected from	Chemical (Concentration)[b]	Type of Protectant	Degree of Protection, % [c]	Reference
Bean, cultivar Pinto	Oxidant	K-Ascorbate (0.01 M)	Antioxidant	52	170
Petunia	Oxidant	K-Ascorbate (0.01 M)	Antioxidant	39	170
Tobacco	Oxidant	Zn-ethylene bis dithiocarbamate dust (variable)	Fungicide	44	30
Tobacco, cultivar White Gold	Oxidant	Phygon XL (variable)	Antioxidant	89	575
Tobacco, cultivar White Gold	Oxidant	Phygon XL (variable)	Antioxidant	78	575
Tobacco, cultivar White Gold	Oxidant	4,4-Dioctyldiphenylamine in butyl latex	Antioxidant	100	502
Bean, cultivar Pinto	Oxidant	Zineb (normal use)	Fungicide	91	277
Bean, cultivar Pinto	Ozone	Zineb (normal use)	Fungicide	97	277

Table 11-26 (Cont.)

Protection of Plants from Oxidant Injury by Application of Protective Chemicals[a]

Plant Species	Pollutant	Chemical (Concentration)	Type	Degree of Protection, %[c]	Reference
Azalea	Oxidant	Benomyl (drench 60 ppm)	Fungicide	96	386
Bean, cultivar Pinto	Ozone	Carboxin (2.3 ppm in soil)	Fungicide	95	458
Radish	Ozone	N^6-Benzyladenine (30-ppm spray)	Growth substance	100	3
Poinsettia	Ozone	Ancymidol (100-ppm spray)	Growth retardant	100	68
Poinsettia	Ozone (chronic)	Benomyl (500 ppm drench)	Fungicide	57	342
Bean, cultivar Pinto	Ozone	Folicote (0.5% spray)	Wax emulsion	92	435
Bean, cultivar Pinto	Ozone	Benomyl (5 ppm in nutrient solution)	Fungicide	97	433
Bean and Cucumber	Ozone	Benomyl (80 ppm in soil)	Fungicide	94	433
Grape	Ozone	Benomyl (6.7 kg/ha 6 times)	Fungicide	53	276

Table 11-26 (Cont.)

Protection of Plants from Oxidant Injury by Application of Protective Chemicals[a]

Plant Species	Pollutant	Chemical (Concentration)	Type	Degree of Protection, % [c]	Reference
Bean, cultivars Tempo and Pinto	Ozone	Benomyl (0.25-0.36%, 4 weekly sprays)	Fungicide	75	334
Bean, cultivars Tempo and Pinto	Oxidant	Carboxin (10% granular as soil amendment, 8 g/5-m row)	Fungicide	100	334
Tobacco	Ozone (0.50 ppm, 2 h)	Piperonylbutoxide (2 mM solution)	Insecticide	99	288
Tobacco	Ozone (0.35 ppm, 2 h)	Safroxane	Insecticide	76	288
Bean, cultivar Tempo	Oxidant	Benomyl (spray 0.24%)	Fungicide	32-41[d]	343
Grass, annual blue	Ozone (0.25 ppm, 2 h)	Benomyl (60-ppm amendment)	Fungicide	85	384
Bean, cultivar Pinto	Ozone (0.30 ppm, 4 h)	Triarimol	Fungicide	81	479
Bean, cultivar Pinto	Ozone (0.25 ppm, 4 h)	Benomyl (160-µg/g soil amendment)	Fungicide	98	434
Bean, cultivar white	Ozone (0.13-0.50 ppm, 0.5 h)	Ascorbic acid	Antioxidant	75	105

Table 11-26 (Cont.)

Protection of Plants from Oxidant Injury by Application of Protective Chemicals[a]

Plant Species	Pollutant	Chemical (Concentration)	Type	Degree of Protection, % [c]	Reference
Petunia	Oxidant	SADH (0.5% spray)	Growth Retardant	82	67
Tobacco	Oxidant	Benomyl (25-ppm drench)	Fungicide	68	503
Tobacco	Oxidant	Benomyl (0.18% spray)	Fungicide	59	446
Tobacco	Ozone	Peroxidase (0.10 ppm injected)	Enzyme	89	37

[a] Selected data from the references cited.

[b] These are applied as sprays unless otherwise noted.

[c] Percent reduction in plant injury from ozone as a result of the protectant treatment.

[d] Increase in yield by protectant application.

poinsettia,[342] pinto bean,[434,561] chrysanthemum,[284] and annual bluegrass.[384] Soil amendments at 2-160 ppm were effective for pinto bean,[341,433,434,469,479] annual bluegrass,[384] and cucumber.[433] Taylor and Rich[503] reported that a single soil amendment at about 25 ppm protected the first eight leaves of tobacco, but not the younger leaves. They also found less tobacco root cyst nematodes from benomyl applications, and the fungicide was not toxic to the plants at the rates used.[433] Benomyl does not induce stomatal closure,[503] so an internal physiologic mechanism must be involved. The active ingredient is the benzimidazole moiety,[434,561] which inhibits senescence in bean and also inhibits the loss of free sterols in plant membranes.[561] Sterols themselves are effective inhibitors,[493] and the mechanism of action may involve effects on membrane sterols and thus membrane permeability. Spotts et al.[492] studied the effects of benzimidazole and ozone on several water relation effects that suggest that the protection is due to the effect of benzimidazole on the cell membrane.

The fungicide Carboxin has received some study, but with varied results. A 0.43 mM solution gave protection at 5-10 days in white bean,[98] a 0.36% spray gave no protection to pinto or tempo bean,[334] and a 0.01% spray increased injury to azalea.[386] Soil applications of Carboxin were generally more beneficial, but results were still varied. A Carboxin soil amendment was phytotoxic to tobacco[503] and pinto bean,[458] although protection was noted in pinto bean. Pinto and tempo bean were protected from oxidant for 36-40 days after a soil amendment with Carboxin at 8 g per 4.6-m row,[334] and the sulfoxide analogue gave protection to white bean, with a 13% yield increase.[99] A soil drench gave protection to soybean, cotton, tomato, and

tobacco at 9.5 ppm,[458] but a soil drench at 1-100 ppm increased senescence in azalea due to oxidants.[386] These differences in species or conditions of application, but they do call for clarification of the possible role of this fungicide (Carboxin) in protection against oxidants. Carboxin had no effect on stomatal opening,[458] and its protective action appears due to the sulfoxide breakdown product,[99,458] which is not fungistatic. Thus, although it can serve a dual role, the modes of action are dissimilar.

In addition to the compounds named, several others have been studied briefly. Cathey and Heggestad[68] found protection to eight cultivars of poinsettia after treatment with the growth retardant Ancymidol and Chlormequate. They also reported a 75% protection in petunia treated with the growth retardant succinic acid 2,2-dimethylhydrazide (SADH) as a 0.5% spray.[67] This was part of a screen of 65 petunia cultivars to ozone. They found that the resistant cultivars did not respond to SADH; this suggested that protection may be related to modified leaf structure (reduced cell size, intercellular space and stomata, and thicker cell walls). The SADH treatment was not effective above an ozone concentration of 0.45 ppm for 3 h. Adedipe and Ormrod[3] found that a 30-ppm spray of the growth hormone N-6-benzyladenine protected radish from ozone at 0.25 ppm (4 h), and Fletcher *et al.*[166] protected white bean by use of a 1-ppm abscisic acid (ABA) solution. The ABA caused partial stomatal closure, which the authors suggested is the mechanism of protection. Studies with ascorbic acid on white bean,[105] the insecticide piperonylbutoxide on tobacco,[288] road dust on pinto bean,[573] and a wax emulsion (Folicote) on pinto bean[435] all stressed that many compounds can act as protectants to vegetation against oxidant (ozone) effects.

Larkin[296] injected several peroxidases at 0.1-10 ppm into one half of a tobacco leaf and found some protection. He suggested that peroxidase, which is often associated with plant stress conditions, may be important in physiologic resistance. It is doubtful that any one mechanism of action exists. It is important that we understand the mechanism of ozone injury and resistance in plants, so that we can determine better what chemicals may play a role in protecting plants against oxidants.

We do not yet have good answers to four basic questions that have kept protective chemicals from practical use: we do not know the frequency or rate of application needed for continued resistance, or, therefore, the total cost of application. We have little idea of the specificity of selected chemicals on different plants. Little is known of possible undersirable residues or side effects. We do not have sufficient prediction accuracy for high-oxidant days for chemicals that are not long-lived. These basic questions need to be answered as chemicals are tested and promoted for use. The ideal chemical would be effective on several species (cultivars), have multiple uses (e.g., fungicides), have a long life (a single preemergence soil application would be best), and leave no toxic residues. Such chemicals would have value, at least until breeding programs can incorporate resistance to pollution stress into new cultivar introductions.

Cultural practices and land use. Ormrod and Adedipe[415] have developed an excellent presentation on edaphic and climatic factors that play a role in making plants more resistant to ozone and other oxidant stress. They suggest how these environmental factors may be used in cultural and management practices to help to alleviate the effects of oxidant pollutants.

These practices may be of help in greenhouse management and to some extent in field irrigation systems. The association of water stress with resistance to oxidant has long been recognized, and growers have been urged to keep this in mind during periods of high air pollution potential. The increased use of carbon dioxide in greenhouse management would have a positive effect, but the additions are made during cooler months, when oxidant concentrations are not high. These practices are worth considering, but are as yet of no more than supplemental help.

Dochinger[124] was able to predict chlorotic dwarf on seedbed white pine with greater than 90% accuracy. Taylor[501] found that susceptible and resistant tobacco could be identified in seedbeds. Both suggested that, for some sensitive plants, time could be saved by visual screening of transplant beds and the use of only the more resistant members of the population as transplants.

Interest in land use planning--with respect to areas to be set aside for agricultural use, as opposed to industrial use--needs to be considered as a potential ameliorating factor in the control of air pollution effects on vegetation. Land use might not be as effective for the oxidant pollutants, because of their ubiquitous nature. Heck and Brandt[211] have developed a brief but acceptable point of view in terms of the need for land use planning and protection of agricultural commodities from air pollution.

A rational approach to land use planning for air quality maintenance may be built on the basis of diffusion modeling. Although the photochemical versions of the models that relate air quality to emission are only beginning to be applied, there has been sufficient validation to permit their use at least to obtain relative assessments (in contrast with prediction of absolute pollutant concentrations). Chapter 5 discusses the principles and performance

of models that can be used for studying the distribution of photochemical ozone and other oxidant concentrations. The application of these techniques requires a succession of forecasts, beginning with land use patterns and continuing with transportation network usage, vehicle emission intensities, stationary-source contributions, and, finally, patterns (in space and time) of pollutant input to the atmosphere. Meteorologic characterizations based on detailed weather data provide the transport measures needed. For various scenarios, the model generates statistics on the air quality that should be expected. The loop is closed by adjusting the land use to achieve the desired atmospheric pollutant concentrations.

RESPONSES OF NONVASCULAR GREEN PLANTS

Lichenologists have long used the presence and abundance of lichen and moss species to map the biologic impact of large urban and industrial areas.[164] Early workers considered changes in the presence and abundance of these organisms to be related to such factors as temperature and humidity. More recently, most researchers have tended to relate changes in lichen population more to industrial air pollution than to other environmental changes. Although most of the air pollution work lacks ambient air quality measurements, there is strong indication that both the presence and the abundance of lichen and moss species are correlated with sulfur dioxide concentrations in large urban and industrial areas. Such studies have not been done in areas where oxidants are the primary air pollutants, nor in rural areas with relatively high oxidant and mixtures of sulfur dioxide and nitrogen dioxide. Thus, little is known about the direct effects of ozone or other oxidants on lichen and moss morphology or physiology.

Comeau and Le Blanc[78] found that a 4-h exposure of Funaria hygrometrica to ozone at 0.25-1.00 ppm stimulated the regenerative capacity of the moss leaves. This was not true for 6- and 8-h exposures.

Glater[178] reported oxidant injury to several species of fern in the Los Angeles area. Initially, tan lesions appeared near the smaller veins, but in no special pattern. Later, the entire leaf became necrotic. Symptom development and sensitivity of leaves were different from those noted in vascular plants. All leaves appeared to be equally sensitive, except for the growing tip and the youngest uncoiling leaves. Occasionally, a young plant was killed.

We know little about the overall effects of ozone or other oxidants in the nonvascular green plants. Although they may not be economically important, such effects may have an adverse ecologic impact.

RESPONSES OF MICROORGANISMS

Heagle[196] reviewed the effects of ozone on fungus growth, sporulation, and germination. Ozone may inhibit colony growth on artificial media, but rarely causes death, even at high concentrations. Differences in species susceptibility are known. Exposure to ozone at 0.10 ppm for 4 h stopped conidiophore elongation and spore production in Alternaria solani.[455] In several fungi, exposure to ozone at 0.10 or 0.40 ppm for 4 h caused a 10-fold to 25-fold increase in sporulation.[196] Heagle[197] reported effects on three obligate fungi from low ozone exposures. Spore germination was not affected in any of these studies. Kuss[292] grew 30 representative fungi on agar and often found increased spore production after exposure to ozone. Spore germination was decreased in most species, but increased in others.

Rabotonova[442] exposed two species of yeast to ozone: Candida lipolytica was sensitive, and C. auilliermondii was resistant. The biocidal activity of ozone was determined under various cultural conditions with air streams of about 150 or 5,500 ppm (v/v for 10-30 min). Ozone was an effective biocide under most conditions, and effectiveness increased with decreasing pH. Kanoh[270] found that exposure to ozone at 30 ppm for 30 min increased oxygen uptake in slime mold (Physarum polycephalum) homogenate. The ozone also increased succinoxidase activity and inhibited part of glycolysis. deKoning and Jegier[118-121] reported effects of ozone on Euglena gracilis that included reduction of net photosynthesis, increase in respiration, and effects on pyridine nucleotide reduction and phosphorylation. They reported that reduction of net photosynthesis was a logarithmic function of ozone concentration in 1-h exposures.[118] They also found a 5% reduction in oxygen evolution after a 1-h exposure to 0.5-ppm ozone bubbled into 5 ml of solution and an additive effect with a mixture of sulfur dioxide and ozone.[121] Verkroost[574] carried out a detailed study of the effects of ozone on Scenedesmus obtusiusculus, Chod. with special concern over the effects on photosynthesis and respiration. A major weakness in this study was the use of an air stream containing ozone at 150 ppm. The report suggested that the primary site of ozone action is the membrane structure, which produces changes in photosynthesis and respiration.

Ozone at high concentrations has been used in a variety of applications for the control and suppression of fungi and bacteria. These applications have included food protection, drinking-water purification,[182] and treatment of sewage.[281,350] It is generally accepted that ozone is not an effective germicide at concentrations below the point of human sensitivity--

0.04 ppm. The germicidal effectiveness depends on concentration, relative humidity, and the specific organism. In many cases, even a concentration of 3-5 ppm was not sufficient to kill some bacteria. Burleson et al.[58] showed inactivation of several viruses and bacteria after ozone exposure and a greater inactivation with simultaneous sonication. Zobnina and Morkovina[597] related the tolerance of a carotinoid strain of Mycobacterium carothenum with the presence of the pigment. The dwarf mutant was much more sensitive.

Large doses of ozone may inhibit growth and sporulation of fungi on fruit, although most fungi tested were resistant to ozone. Spaulding[490] reported that ozone acted as a surface biocide. Above 0.5 ppm, ozone inhibited surface growth of fungi on strawberry and peach. At 0.06 ppm, it severely injured leaves on head lettuce after 8 days. He also reported some direct injury on peach fruits above 0.5 ppm. Ridley and Sims[463] extended the shelf-life of strawberry and peach by exposing them to ozone, but stated no concentrations. Ozone at 1-2 ppm for 1-2 h/day controlled the surface growth of fungi and sporulation on apple, reduced offensive odors, and decreased the ripening rate.[489] Watson[579] found that ozone at 0.4-2.0 ppm acted as a surface fungicide, but did not penetrate. Thus, there was no effect on fungal growth within the fruit. Sporulation and some decay control of Penicillium digitatum and P. italicum were noted in open storage boxes of lemon and orange exposed to ozone at 1 ppm.[92] Ozone was more effective than a fungicide dip in controlling Botrytis bud rot of gladiolus, but no concentrations were determined.[329] In general, researchers have suggested that rather large dosages of ozone are required to protect storage fruits from fungal infection. These concentrations may be so high as to

preclude the use of ozone in storage facilities. The ability of ozone to reduce spore germination in fungi apparently depends on species, spore morphology, moisture, and substrate.[196] Single-celled spores and those with thin cell walls are most sensitive. Wet spores are more sensitive than dry spores.

Haines[185] reported that ozone at 4 ppm retarded growth of Escherichia coli, whereas 10 ppm prevented growth. Scott and Lesher[476] found that approximately 2×10^7 molecules of ozone per bacterium killed 50% of the cells of E. coli and that the primary effect was on the cell membrane. Only metabolites leached from the cell were affected. Elford and van den Ende[140] reported that ozone at 20 ppm had a lethal effect on some bacteria deposited from aerosol mists on various surfaces. Relative humidity is an important factor, particularly when ozone concentration is low. They found little death at a humidity below 45%, at concentrations of 1 ppm, as opposed to a 90% kill in 30 min at 0.025 ppm with a humidity of around 70%. A 5-min exposure of Bacillus cereus to ozone at 0.12 mg/liter was the minimal lethal dose, whereas 0.10 mg/liter was effective for B. megaterium and E. coli.[56] Spores of the Bacillus sp. were killed by ozone at 2.29 mg/liter. These responses were of the all-or-none type with ozone between 0.4 and 0.5 mg/liter of water. Time of exposure, from 1 to 32 min, was not important. Chlorine was effective at 0.27-0.30 mg/liter, with time an important consideration. These two gases did not affect E. coli in the same way.

In most research on lower organisms, there has been an attempt to use ozone as a germicide or to understand the interactions of pollutants and pathogens on the responses of higher plants. In few studies has the interest been on the effects of ozone on the organisms themselves, except in the studies of effects on algae.

BIOLOGIC MONITORS

The use of biologic indicators as an early detection system for severe air pollution episodes or for chronic air pollution problems is of interest to many urban and industrial control officials. Plants served as air pollution detection systems long before it was acknowledged a problem by industry or government. Higher plants serve as useful detection tools, because they develop characteristic symptoms from acute exposure, even though the symptoms are not necessarily specific to cause. Cause and effect can be reliably ascertained, when an air pollution source is identified and symptom patterns are identified within given sensitive species.

Plants have been used effectively in field surveys to determine the extent and magnitude of pollution problems and in bioassay techniques in conjunction with field surveys. Most general review articles (Table 11-1) treat, to some extent, the use of plants as indicators of air pollution. Went[585] covered plant sensitivities to pollutants and the use of plants as indicators. He stressed the photochemical oxidants and recommended charcoal filtration for greenhouse use. Heck[209] presented a detailed discussion and review of plants as indicators in field surveys and in the bioassay of photochemical problems. Heggestad and Darley[222] reviewed plants as indicators of ozone and PAN and recommended pinto bean as the best indicator of both oxidants. They also suggested three variably resistant tobacco cultivars for use in monitoring the severity of ozone episodes.

It may be of value to differentiate the indicating and monitoring uses of plants. Most reported work has used the indicator concept, with plant injury (symptoms) as indicative of a problem. Monitoring implies some degree of reliability. Several studies have attempted to use plant response

as a monitor of pol lution concentrations or doses, with variable but uncertain success. Plants could also be considered monitors if they gave a reliable index of the biologic effects on biologic systems of concern to man (crops, forests, animals, and man himself). The latter has not been seriously discussed.

This section is divided into reviews of plants in field surveys and plants as a bioassay technique and a brief discussion of the possible value of biologic indicators.

Field Surveys

Field surveys have played a significant role in the assessment of air pollution problems. Basic techniques were developed in surveys around point sources of sulfur dioxide and fluoride. Similar techniques have been developed regarding photochemical pollutants, starting with the early report by Middleton et al.[369]

Treshow[565] has developed the thesis that foliar injury is a useful criterion in the identification and analysis of air pollution effects on vegetation and presented some basic concepts for use in field evaluation.

Sharma and Butler[482] found that white clover in a highly polluted area (Nashville, Tenn.) had a lower stomatal frequency, a higher trichome frequency, and a greater trichome length than the same species growing in an area of lower pollution. They suggested oxidants as one of the pollutant stressors. It is possible that pollutant stress (among other things) is causing selective pressures that favor these changes; thus, a separate race may be evolving. This type of work needs further evaluation and exploration.

Most researchers have felt that biochemical changes in plant tissues are associated with so many normal growth and stress phenomena that they

would have no relevance in field evaluations of air pollution effects. Keller has associated changes in peroxidase activity with different pollution stresses (flouride and oxidants) in apricot and white ash growing in areas of high pollution, as opposed to those of low pollution. This study suffers from lack of pollution monitoring (although leaves were analyzed for flouride) and consideration of other stress factors. This is the same type of criticism that has long been made of lichen studies in industrialized areas.[164] Lichens may respond to the photochemical complex, although such work has not been reported. The larger question of the value of these studies persists. If, in fact, changes in some biochemical entity could be clearly associated with one or more pollutant and if this could be separated from other stresses, what would it tell an interested party? Can it be related to adverse yield, quality, or genetic changes? These approaches to understanding air pollution effects through field surveys appear to be of doubtful value and must rely on statistical methodology involving many variables.

A technique that offers greater potential is the use of remote sensing of injury to vegetation. This technique was first tried with ponderosa pine as the species of concern.[586] Wert et al.[587] and Larsh et al.[298] found that injury to ponderosa pine in the San Bernardino Mountains was severe enough for the technique to have real potential. They were able to identify the severity of diseased areas through color photography with a high degree of correlation with ground plots. In severely affected areas where air pollution is the principal stressor, this approach offers a rapid and inexpensive technique for surveying large tracts. It is not yet possible to use remote sensing to identify pollutant stress amid a variety of other stressors. However, as these techniques are improved, they should be applied to air pollution problems.

Early field surveys depended on identification of a syndrome of responses that included symptoms on both native and cultivated plant species. Middleton and Paulus[366] directed the first large-scale survey to determine the extent and severity of photochemical oxidant effects in California on crops of agronomic importance. They delineated four categories of crops (field, flower, fruit, and vegetable) and one of weeds. This was the most extensive survey of oxidant effects until the late 1960's. The information was later used as a basis for subjective estimates of economic losses. This type of visual assessment of foliar injury has been attempted in many states and has been purposefully developed in some for use in economic estimates of damage to vegetation.

Plant Bioassay of Oxidant Effects

During the 1950's, when the phytotoxic components of the photochemical oxidant complex were not known, plants were used extensively as a bioassay of many simulated reaction mixtures to indicate toxicity.[183, 209, 238, 406, 408, 496]

Plants were also used to help in identifying specific components of photochemical reaction products, such as PAN and its analogues.[496]

This bioassay technique was also used to determine the phytotoxicity of the ambient photochemical complex and to attempt to standardize plants, so that oxidant concentrations could be determined from severity of injury. Two such studies were initiated at about the same time in the Los Angeles basin. Middleton et al.[368] established five stations where young pinto bean plants, grown under uniform conditions, were set out daily and foliar injury intensity was compared with results from oxidant instruments. Results were recorded as the percentage of days showing plant injury and the average injury index per day per station. There was a relationship between injury

and oxidant values, but the correlations were not significant and the association was obscure. The most detailed study was carried out over a period of about 4 years with annual bluegrass.[407] The plants were grown under standardized conditions,[268] and injury development was concisely defined.[37] The plants were transported daily to locations around Los Angeles County, California. They were exposed for 24 h and returned for a control posttreatment period before injury assessment. The injured leaf areas were measured and correlated with the severity of oxidant episodes. The procedures were time-consuming, and the results were poorly correlated with oxidant concentrations. Thus, this procedure was discontinued as automatic instrumentation became available.

The bioassay technique was developed to reduce the uncertainties associated with the use of native vegetation or cultivated crops. Plants can be started under controlled conditions and exposed under standardized conditions. Species and cultivars can be selected for oxidant sensitivity and symptom characteristics. The two studies just noted were the most closely controlled. Similar work has not been repeated. However, many investigators have grown plants under known cultural conditions and then transplanted them to field sites where they received special care. These plants can then be read for foliar symptoms throughout a given period, and the symptoms related to oxidant concentrations. The lack of apparent correlation in the two early studies could be due to the lack of specificity for the monitored oxidants, the presence of different concentrations of interacting oxidants at different times, or variations in cultural conditions between exposure times.

Brennan et al.,[50] using petunia, found a better correlation with atmospheric aldehydes than with total oxidants. This appears spurious,

because researchers do not regard aldehydes themselves as important atmospheric phytotoxicants. Macdowal et al.[324] were able to correlate tobacco injury with oxidant concentrations by considering ozone flux into plant leaves. They then predicted fleck attacks with fair consistency on the basis of meteorologic considerations. Several investigators have used acknowledged plant sensitivity and emphasized the importance of accurate monitoring for pollutants[84] and meteorologic factors.[123] These aspects must concern any investigator who is seriously considering a plant monitoring system. Berry[26] developed clonal white pine material for use with specific atmospheric pollutants. Oshima[421] has recommended pinto bean as a reliable monitor of oxidant intensity with a subjective injury evaluation (0-5) that has been standardized against colored photographs. This is not as sensitive as a percentage separation, but is no doubt easier for an untrained observer to use. He did show good correlation between weekly oxidant concentrations and plant response. This a relatively simple system, but the monitoring plant is not as sensitive to low concentrations of oxidant of oxidant as is Bel W[3] tobacco.

The most extensively used monitoring system for photochemical oxidants has used the ultrasensitive Bel W[3] tobacco.[224] The technique was first developed by Heck and associates.[213, 220] The response of this tobacco variety was used to determine the distribution, frequency of occurrence, and approximate average concentrations of photochemical oxidants in and around Cincinnati, Ohio, over four growing seasons. Monitoring sites were set up at various distances from Cincinnati, up to 75 miles (120 km) due east. Injury responses from this monitoring network showed that the average effective concentration of photochemical oxidants was as high in rural Ohio as in Cincinnati. Although

oxidant concentrations did not correlate well with plant injury on a weekly basis, the seasonal ratio of injury to oxidants was similar over a 3-year period. It was suggested that all areas east of the Mississippi have sufficient photochemical oxidants to cause injury to sensitive plants at some stage of their development. This monitoring system has since been used as part of a regional study to determine the importance of oxidant pollutants with respect to sensitive crops in the northeastern United States,[262] and similar systems have been used as oxidant indicators in South Dakota,[176] Germany,[285] the United Kingdom,[18] and other locations--including North Carolina, Washington State, and Arizona. This type of monitoring system was designed to provide communities with estimates of the frequency of occurrence of phytotoxic concentrations of oxidants, of the relative severity of each episode, and of the regional distribution of oxidant pollutants. The system will not give a reliable estimate of oxidant concentrations. Thus, it is an indicator of oxidant phytotoxicity, instead of a monitor for oxidant concentrations.

Craker et al.[96] used Bel W 3 tobacco as a relatively simple monitoring device whereby interested citizens in a community could develop some idea of the biologic severity of oxidant pollution in their community. There are problems with their study, but the concept is useful and might alert a community to potential pollution problems.

Several investigators have used systems ranging in complexity from simple antioxidant-impregnated cloth covers[578] to fairly elaborate filtered and unfiltered chambers[145, 204, 228, 237] to determine the effects of ambient oxidants on sensitive indicator (monitoring) plants. Bel W 3 tobacco was used in all these tests. Such tests can be used to determine growth and yield reductions, as well as to identify possible problems.

Discussion

Many investigators have discussed the use of biologic indicators as early detection systems for air pollution problems. Some have viewed the possibility of a nationwide monitoring system for air pollutants and other environmental contaminants. Others have felt that the increased collection of chemical data at many sites obviates biologic indicators or monitors. However, the chemical and meteorologic data do not yet give a clear picture of the biotoxicity of air pollution episodes. Thus, agencies should give serious consideration to the development of national networks. If such interest becomes widespread, it should be possible to correlate the biologic response of the monitors with the response of important economic species, ornamental species, or some human health measures. Without the ability to correlate these factors, bioindicators are merely another index of pollution that carries no rational value. Without the use of the bioindicator, we are back to a direct assessment of the effects of a given pollution episode on the biologic organisms of interest to man.

ECONOMIC ASSESSMENT

This section will bring into focus our present understanding of the economic effects of oxidant air pollution on vegetation. Heck and Brandt[211] presented in depth the problems inherent in making an economic evaluation of the response of plants to air pollutants, including ozone and other oxidants. They used the distinction between injury and damage that was proposed by German workers.[41] Injury is defined as any identifiable and measurable response of a plant to air pollution; damage is any measurable adverse effect on the desired or intended use of the plant. Thus, before an effect

on a plant can be evaluated in terms of economics, the plant must have been altered either quantitatively or qualitatively in such a way as to reduce its use value. In this context, visible symptoms or transient changes in physiologic responses may not result in an economic loss. Thus, leaf necrosis in soybean is injury; to be classified as damage, the injury must affect yield. In the same way, a severe oxidant episode that bronzes the leaves of romaine lettuce may not affect biomass, but will affect use and may result in complete economic loss. Ornamental plants, whose major use value depends on appearance, may be both injured and damaged.

Emergence tip burn of white pine [23] and other physiogenic diseases of white pine associated with air pollution occur throughout the natural range of the species. Insular stands containing ponderosa pine are frequently injured in the Southwest. The significance of these physiogenic diseases is not understood because they affect only sensitive genotypes. The disease occurs randomly in the forest, and its most obvious effect is the slow selection and gradual elimination of genotypes that may have otherwise superior silvicultural characteristics. This could be a serious loss to future tree improvement efforts and may be occurring in other forest species. Economic considerations have not been addressed in any reasonable way.

Early work with sulfur dioxide showed a linear relationship between visible injury and reduction in yield for many crop species. The assessment was made that no reduction in yield would be found unless visible injury were noted. Definitive research with ozone, other oxidants, or mixtures of these pollutants with other gases has not been done. Thus, we do not know whether such relationships between visible injury and yield hold for the oxidants, but data in Table 11-3 suggest that for acute exposures there may

be good correlations between injury and yield reductions. Many researchers have hypothesized that the oxidants may have an effect on plants that will produce a yield reduction with little or no visible injury. Such studies need to be designed in a more definitive manner before it is concluded that yield reductions without visible symptoms are clearly acceptable. Projections of yield losses have made use of some of the data reported earlier.[19,370]

Oshima[419,420] has developed a methodology for evaluating and reporting economic crop losses that involves continuous air monitoring, chamber exposures, and monitoring plant species. He has attempted to weld these into a comprehensive method of determining yield reductions. He has reported yield reductions for some species, but has not fully clarified the procedure. He presented no economic values in either report. This type of approach needs to be considered further.

The reports of specific effects related to sensitive growth stages,[540, 542, 543, 545] especially to reproductive growth stages in which the pollutant may be affecting reproductive structures and not leaf tissues,[62, 63, 155] are probably of greater importance to the understanding of reduced yield without visible symptoms than to the potential of a debilitating effect caused by chronic continuous exposure to ozone or other oxidants. These economic considerations have not been addressed.

Any attempt to assess oxidant damage to agricultural crops requires judgment by a competent investigator. Landau and Brandt[294] believe that the success of crop surveys is directly related to the number of subjective decisions required in data collection. The first surveys were conducted in California[366, 369] and were used, with a general survey of conditions on the East Coast, to develop some of the early economic estimates of $8-10 million

in California and $18 million on the East Coast. These estimates were raised to $500 million on the basis of increased awareness of pollution effects and increased recognition of additional sensitive species. However, all early estimates were subjective, with no substantial backup data.

Since 1969, a number of states have instituted an intensive training program for county agricultural agents and made detailed reports of crop injury and damage [163, 292, 295, 370, 399, 429] (Table 11-27). The first such report came from Pennsylvania [295] in 1969 and gave an estimate of a $9.6 million loss to agronomic commodities from oxidant pollutants. This survey included direct and some indirect cost. Similar surveys have been conducted in New England, [399] New Jersey, [163, 429] California, [370] and Michigan (no report is currently available). These surveys considered yield reductions on the basis of injury and made no direct growth or yield assessments, although subjective estimates of damage were obtained. Pell and Brennan [432] presented a well-developed thesis on the rationale for the difference in estimated losses to agriculture in New Jersey between 1971 and 1972. They discussed this in relation to the overall problem of assessing agricultural losses.

A survey was initiated in 1969 [19] by Stanford Research Institute (SRI) to develop an empirical model for assessing damage to vegetation. This program made use of laboratory and field data from controlles exposures on various crops and chemical data from simulated reaction chambers, so estimates of ozone and other oxidants could be made on the basis of concentrations of primary pollutants. Hydrocarbon was chosen as the basic pollutant from which to develop the model for prediction of expected oxidant values and, therefore, effects on various crops. From

Table 11-27

Economic Estimates of Crop and Vegetation Losses in the United States Owing to Oxidant Air Pollutants[a]

Area and Year	Cost $1,000	Comments	Reference
United States, 1963	65,000	First approximation for commercial crops (SRI)	19
	121,400	Revised SRI report to include ornamentals	19
California, 1963	33,700	Revised SRI report	19
1970	17,500	Does not include ornamentals or indirect costs	370
Pennsylvania, 1963	6,300	Revised SRI report	19
1969	9,600	Pa. survey; includes indirect costs	295
1970	60	Pa. survey, as above	293
New Jersey, 1971	960	N. J. Survey of a limited number of crops, based on visible injury	163
1972	60	N. J. as above	429
New England, 1971	1,100	Mass. survey; primarily crops and ornamentals	399

[a]Some of variation reflects methodology; other reflects differences in plant susceptibility and pollution over years.

the oxidant value, injury and damage for specific crops were calculated for over 100 statistical reporting areas in the United States. The report used many subjective assumptions and was related primarily to visible injury symptoms. The results were preliminary, and the approach had many deficiencies; but similar approaches should be contemplated in the development of damage functions that will give some reliable estimates of economic losses. On the basis of the SRI model, the 1969 estimated loss to vegetation from oxidants in the United States was approximately $125 million. Considering the increase in crop values and assuming that oxidant concentrations have not been significantly reduced during the last few years, the loss in 1974 from oxidants could approach $300 million, on the basis of the SRI study.

A summary of estimates derived from various surveys and assessment techniques is shown in Table 11-27. These values are suggestive at best. As with all values developed for agricultural losses, these have been directed at losses to the producer--not to the consumer.

Whereas a reduction in production may actually increase the aggregate farm income and produce serious income distribution problems, the consequent reduction in marketable surplus would cause a significant rise in the cost to consumers, because of the inelastic consumer demand for most agricultural crops. Therefore, at the consumer level, losses based on farm prices are not appropriate and are likely to be conservative. Because of percentage markups and fixed wholesale and retail marketing costs, the cost to the consumer of agricultural losses could be twice as great as that observed at the farm level--i.e., a $300 million loss at the farm level in 1974 could represent a $600 million loss to the consumer.

SUMMARY

Oxidant injury to vegetation was first identified in 1944 in the Los Angeles basin. Our understanding of oxidant effects and of the widespread nature of their occurrence has increased steadily since then. Although the major phytotoxic components of the oxidant (photochemical) complex are ozone and peroxyacetylnitrate (PAN), indirect data support the contention that other phytotoxicants are present in the photochemical complex. Ozone is considered the most important phytotoxic component and was first identified as the specific cause of weather fleck on tobacco and stipple on grape. PAN is associated with the undersurface glazing and bronzing associated with many of the vegetable crops.

Plant response to oxidants (including ozone and PAN) is often divided into visible and subtle effects. Visible effects are identifiable pigmented, chlorotic, and necrotic foliar patterns resulting from major physiologic disturbances. Subtle effects produce no visible injury, but include metabolic disturbances and may be measured on the basis of growth and long-term biochemical changes. These effects may influence plant populations and communities and could have an adverse influence on ecosystems. Visible injury may be acute or chronic. Acute injury breaks down the cell membrane and causes cell death, with leaf necrotic patterns that may be characteristic for a given oxidant, but can be confused with other stress factors. Classic injury from ozone is the upper-surface fleck on tobacco and the stipple of grape. Many plants show an upper-surface bleach with no lower-surface injury. Bifacial necrotic spotting is common and may appear flecklike. Classic injury from PAN appears as a glaze followed by bronzing of the lower leaf surface in many plants. Complete collapse of leaf tissue can

occur, if concentrations are high. Chronic injury is associated with disruption of normal cellular activity followed by chlorosis or other color or pigment changes that may lead to cell death. Chronic injury patterns are generally not characteristic and may be confused with symptoms caused by biotic diseases, insects, nutritional disorders, or other environmental stresses. Early leaf senescence and abscission may result from chronic exposure.

Leaf stomata are the principal entry sites for ozone and PAN. Stomata, when closed by any of a number of factors, will protect plants. Ozone and PAN may interfere with various oxidative reactions in plant cells. Membrane sulfhydryl groups and unsaturated lipid components may be primary targets of oxidants. Physiologic leaf age is an important consideration in the response of the leaf to oxidants. Young leaf tissue is more sensitive to PAN, whereas newly expanding and maturing tissue is most sensitive to ozone. Light is required before plant tissue will respond to PAN, but not to ozone. Oxidants affect such physiologic processes as photosynthesis, respiration, transpiration, stomatal opening, metabolic pools, biochemical pathways, and enzyme systems. The acute response of plants to ozone and PAN may result from a saturation of sensitive cell sites and a disruption of normal cellular repair mechanisms. Chronic injury probably results from secondary reactions involving membrane injury.

There is evidence that ozone is a radiomimetic gas. Ozone affects pollen germination in some species and thus may directly affect yield. A study with Arabidopsis thaliana suggested no mutagenic effects from ozone on this plant over five generations.

Ambient-oxidant studies in filtered versus nonfiltered field chambers have reported up to 50% reduction in citrus yield (orange and lemon), a 10 - 15% reduction in grape yield in first year and 50 - 60% reductions over the following 2 years, and a 5 - 29% reduction in yield of cotton lint and seed in California. Losses of 50% in some sensitive potato, tobacco, and soybean cultivars have been reported from the eastern United States. It is apparent that ambient oxidants do reduce yields of many sensitive plant cultivars. Growth reductions associated with acute exposure to ozone are often associated with injury; sometimes the correlations are high. Even multiple exposures and sometimes chronic exposures have shown fair to good correlation between injury and growth (biomass) reductions. The greater reductions in root growth than in top growth reported in several species are related to solute transport and may be fairly common under some conditions. Ozone affects nodule number, but not nodule size or efficiency in clover, soybean, and pinto bean. This causes a reduction in nitrogen fixation associated with legumes and, if widespread, could have a major impact on plant communities and affect fertilizer needs. The effect on nodulation is related to carbohydrate supply. Yield reductions with little injury after chronic exposure are known for several crops. Severe injury in tomato was required before a yield reduction was found. Chronic exposures to ozone at 0.05-0.15 ppm for 4-6 h/day will produce yield reductions in soybean and corn grown under field conditions. The threshold appears to be 0.05-0.10 ppm for some sensitive cultivars and is well within values monitored in the eastern United States. Growth or flowering effects, at chronic exposures to ozone at 0.05-0.15 ppm for 2-24 h/day are reported for carnation, geranium, radish, and pinto bean grown in greenhouse chambers.

Plant sensitivity to ozone, PAN, and other oxidants is conditioned by many factors. Genetic diversity between species and between cultivars within a species is well documented. The mechanism of genetic resistance is known for only one onion cultivar and is related to the effect of ozone on stomatal closure. Variants within a natural species are well known for several pine species, including white, loblolly, and ponderosa. Plant sensitivity to oxidants can be changed by both climatic and edaphic factors. A change in environmental conditions will initiate a change in sensitivity at once, but it will be 3-5 days before the response of the plant is completely changed. Plants generally are more sensitive when grown under short photoperiods, medium light conditions, medium temperature, high humidity, and high soil moisture. Injury to PAN may increase with increasing light intensity. Conditions during exposure and growth affect the response of plants to oxidants in similar ways. However, plants exposed to ozone are more sensitive to increasing light intensity and, in some cases, to decreasing temperature during the exposure period. In general, growth factors that tend to cause a physiologic hardening of plant tissue make the plants more tolerant to ozone. At the time of exposure, factors that increase water stress tend to make plants more tolerant to ozone. Soil moisture is probably the most important environmental factor that affects response during the normal growing season.

Plants respond in different ways to pollutant mixtures; less than additive, additive, and greater than additive effects have been reported. Mixtures of ozone with sulfur dioxide and of nitrogen dioxide with sulfur dioxide can cause oxidant-like symptoms in some sensitive plants. Mixtures can cause effects below the threshold for either gas, although there is some

disagreement on this in regard to ozone. Ratios of mixtures, intermittent exposures, sequential exposures to pollutants, and predisposition by one pollutant to the effects of a second pollutant may all be important in nature, but little research has been done.

The response of some plants to oxidants is conditioned by the presence or absence of biotic pathogens. Depending on the plant and the pathogen, oxidants may cause more or less injury to a given species. Pathogens may protect their host or make it more sensitive. The pathogens themselves may be injured or may be protected by the host plant. This subject is just starting to be understood.

Oxidant injury to ponderosa pine predisposes the trees to later invasion by pine bark beetles. Ozone and ozone-sulfur dioxide mixtures may decrease the population of soybean nematodes. Both greater and smaller effects have been noted when herbicides have been used in the presence of high oxidant concentrations.

The two most critical factors in terms of air quality standards are duration of exposure and concentration. These two factors determine the exposure dose for a plant. In determining the response of vegetation, concentration is more important than time. A given dose presented to a plant in a short period has a greater effect than the same dose applied over a longer period. This suggests a threshold effect for plant populations and is probably related to the repair mechanisms inherent in biologic systems. Sufficient information is not available from long-term chronic studies, but a threshold between 0.05 and 0.10 ppm is probable for ozone (oxidant). For acute effects, an overall threshold concentration with respect to time can be determined from Figure 11-6 and Table 11-24a. For pinto bean (Table 11-22), this threshold

for injury is about 0.03 ppm for 8 h and about 0.10 ppm for 1 h. This suggests that oxidant standards may be needed for periods up to 8 h.

Vegetation can act as a major sink for oxidants over time, but has a relatively minor effect on oxidant concentrations during episodes of high air pollution, is more effective at some seasons or under some cultural and management practices than others, and should not be considered an important contributor to short-term reductions in oxidant or ozone concentrations.

Plant protection from air pollution stress has involved three types of programs. Several researchers are including pollutant stress in standard breeding programs with the aim of developing resistant cultivars. Our present concepts of pollution effects suggest that the gene pool of all species is large enough to permit the development of more tolerant cultivars. Natural selection will slowly do this for native vegetation. If pollution concentrations go no higher, this should be an effective protection device. Interim measures involve the use of chemical sprays. Such sprays are not yet economically feasible, but several do give adequate protection against oxidants. Fungicides, such as Benomyl, may serve a dual function. Cultural and land use practices may also play important roles, especially on a short-term basis.

Little research on the effects of oxidants on nonvascular green plants and microorganisms has been reported. Lichens and mosses are responsive to acid gases, but there is no definite evidence that they respond to oxidants. Ferns may be especially sensitive, but their injury response is much different from that of higher plants. Growth and sporulation of fungi on surfaces are usually, but not always, affected. Ozone does not penetrate the leaf tissue or the colony and thus does not cause death of colonies. Ozone from

0.1 to several milligrams per liter of solution is required to kill many microorganisms in liquid media. Most work with microorganisms has been done to study the effectiveness of ozone as a biocide in the storage of vegetation or treatment of water or sewage supplies.

Plants have been used as biologic indicators of oxidant pollutants for many years. Attempts have been made to use plants as monitors, but too many unknown variables are involved. Plants may be capable of monitoring the total biologic potential for adverse effects, but no research has been developed along these lines.

Losses based on farm prices are not appropriate at the consumer level and are likely to be conservative. Because of percentage markups and fixed wholesale and retail marketing costs, the cost to the consumer from agricultural losses to oxidant pollutants could be as much as $600 million per year.

RECOMMENDATIONS

These recommendations are not listed in a priority order, but many could be followed in parallel or simultaneously. In general, the recommendations presented by Heck et al.[219] are still germane to research needs in the subject of oxidant pollutants.

A few definitive experimental designs are needed to further our knowledge of acute dose-response information on ozone. Much of this type of information is still needed for PAN and its analogues. All experimental designs should incorporate dose response.

Studies to develop dose-response curves for chronic exposures of crop and native species over growing seasons and under field conditions are needed for ozone, PAN, and other oxidants.

Research should be continued with filtered and nonfiltered field chambers to study effects of ambient oxidants on important agronomic, horticultural, and native species. We know that there is a problem, but its significance and magnitude are matters of conjecture.

There is a critical need to understand the interaction of multiple pollutants on individual plant species and ecosystems. Multiple-pollutant effects are generally important, but little is known of their effects on most plants. Variable concentrations, ratios of pollutants, and age of plants all affect response.

Models should be developed to understand the relative importance of other variables as they affect plant dose response. These include, but are not limited to, climatic, edaphic, biotic, and genetic factors. Considerable

information is available, but there are many gaps, and no comprehensive programs are in progress to determine how these factors act and interact to affect a plant's response.

The mechanism of response and the biochemical systems affected are not understood. Although plant membranes are considered the primary sites of action for the oxidant pollutants, there is no definitive work on this. An understanding of these responses would be supportive of breeding and spray protective programs.

Both breeding and spray protective programs need to be developed, so that better-yielding and better-quality cultivars will be protected against oxidant pollutants.

Some effort is needed to explore the feasibility of using plants to monitor the overall biologic activity (or biomass reductions) caused by photochemical oxidants in specific air basins or regions. The response of sensitive plants should be correlated with the response of plants of economic and aesthetic importance.

Additional monitoring of multiple pollutants is needed in rural areas.

Whenever possible, measures that vary on a continuous scale (e.g., biomass) should be used with subjective estimates of injury (e.g., indexes of visible injury).

APPENDIX TO CHAPTER 11

INDEX OF PLANT NAMES AND REFERENCE NUMBERS[a]

ALFALFA (Medicago sativa, L.) -- 79,233,245,331,447,487,538,547,548

APPLE (Malus, sp.) -- 371,489

APRICOT (Prunus armeniaca, L.) -- 272

Arabidopsis thaliana, L., Britt -- 57

ARBORVITAE (Thuja orientalis, L.)

ASH, white (Fraxinus americana, L.) -- 264,272

ASPEN, quaking (Populus tremuloides, Michx.) -- 194

AVOCADO (Persea, sp.) -- 512

AZALEA (Rhododendron, sp.) -- 386

BARLEY (Hordeum vulgare, L.) -- 22a,202,348,474,477

BEAN (Phaseolus vulgaris, L.) -- 7,22a,69-71,98,99,110,111,133,134,137, 138,141,143,153,170,222,244,247,261,277-279,297,319,327,328,334,341,343,344,345, 348,349,421,424,434,452,457,458,479,495, 496,506,517,551,552,556,573,596

BEAN, lima (P. limensis, Macf.) -- 547

BEET, table (Beta vulgaris, L.) -- 60,369,412

BEGONIA, christmas (Begonia socotrana, Hook, f.)

BEGONIA (Begonia, sp.) -- 214,297

BIRCH, white (Betula alba, L.) -- 563

BROCCOLI (Brassica oleracea, L.) -- 214,547

CABBAGE (B. oleracea, L.) -- 344,547

CARNATION (Dianthus caryophyllus, L.) -- 156,157

CEDAR (Juniperus, sp.) -- 373,507

Chenopodium album, L. -- 194

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Chenopodium fremontii, L. -- 194

CELERY (Apium graveolens, Linn) -- 170

CELOSEA (Celosea, sp.) -- 5

CHARD, swiss (Beta vulgaris, L.) -- 369,560

CHRYSANTHEMUM (Chrysanthemum, sp.) -- 128,297,329,330,593

CITRUS (Citrus, sp.) -- 520

CLOVER, red (Trifolium pratense, L.) -- 297

CLOVER, white (T. repens, L.) -- 286,482

CLOVER, white sweet (Melilotus alba, Desr.) -- 482

COLEUS (Coleus blumei, Benth.)

CORN (Zea mays, L.) -- 63,206,240-242,266,297,397,418,509,570

COTTON (Gossypium hirsutum, L.) -- 53,201,458,531

CRESS (Lepidium sativum, L.) -- 100

CUCUMBER (Cucumis sativus, L.) - 416

Descurainia, sp. -- 194

DUCKWEED (Lemna minor, L.) -- 158

FIR, balsam (Abies balsamea (L.), Mill.)

FIR, Douglas (Pseudotsuga menziesii (Mirb.) Franco.)

FIR, white (Abies concolor (Gord. & Clend.) Lindl.)

GERANIUM (Geranium, sp.) -- 156,336,337

GERANIUM, wild (G. fremontii L. Torr. ex A. Gray) -- 194

GLADIOLUS (Gladiolus, sp.) -- 329,330

GRAPE (Vitis, sp.) -- 273,276,461,518,519,522

INDEX OF PLANT NAMES AND REFERENCE NUMBERS[a]

GRAPEFRUIT (Citrus, sp.) -- 170

GRASS, annual blue (Poa annua, L.) -- 268,384,407

GRASS, bent (Agrostis palustris, Huds.)

GRASS, Bermuda (Cynodon dactylon, L.)

GRASS, brome (Bromus tectorum) -- 441

GRASS, brome (B. inermis, Leyss) -- 297

GRASS, Kentucky blue (Poa pratensis, L.)

GRASS, orchard (Dactylis glomerata, L.)

GRASS, perennial rye (Lolium perenne, L.)

GRASS, red fescue (Festuca rubra, L.)

GRASS, zoysia (Zoysia japonica, Steud.)

GUM, sweet (Liquidambar styraciflua) -- 563

HEMLOCK, Eastern (Tsuga canadensis (L.), Carr.)

HOLLY, English (Ilex, sp.)

IMPATIENS (Impatiens sullanii, Hook) -- 5

LARCH, European (Larix decidua, Mill.)

LARCH, Japanese (L. leptolepis (Sieb & Fucc.) Gord.)

LEMON (Citrus, sp.) -- 92,129,192,521

Lepidium virginicum, L. -- 47,194

LETTUCE (Lactuca sativa, L.) -- 170,369,490,510

LOCUST, honey (Gleditsia triacanthos, L.) -- 127

Madia glomerata -- 194

MAPLE, red (Acer rubrum, L.) -- 563,564

INDEX TO PLANT NAMES AND REFERENCE NUMBERS[a]

MAPLE, silver (A. saccharinum, L.) -- 127,264

MAPLE, sugar (A. saccharuim, Marsh) -- 264,563

MARIGOLD (Tagetes patula, L.) -- 5

MORNING GLORY (Ipomoea purpurea, Roth) -- 400

MUSTARD (Brassica pekinensis)

OAK, red (Quercus rubra, L.) -- 127

OAK, swamp (Q. palustris, DuRoi) -- 127

OAK, white (Q. alba) -- 127,563

OATS (Arena sativa, L.) -- 62,64,186,195,297,477

ONION (Allium cepa, L.) -- 141,142,416

ORANGE (Citrus sinensis, Osbeck) -- 92,192,521

PEACH (Prunus persica, Sieb. & Zucc.) -- 463,490

PEA, cream (Pisum sativum, L.) -- 174

PEANUT (Arachis hypogaea, L.) -- 13,203

PERIWINKLE (Vinca minor, L.) -- 297

PETUNIA (Petunia hybrida, Vilm.) -- 50,91,93,128,170,190,191,193,310, 496,506,510,560,593

PINE, Austrian (Pinus nigra, Arnold)

PINE, eastern white (P. strobus, L.) -- 16,23,26,28,40,82,86,113,124-126,248-250,313,315,590

PINE, jack (P. banksiana, Lamb.)

PINE, Jeffrey (P. jeffreyi) -- 373,507

PINE, loblolly (P. taeda, L.) -- 27,582

PINE, pitch (P. rigida, Mill.)

PINE, ponderosa (P. ponderosa, Laws) -- 77,108,149-151,298,372,374,375, 378-380,426,507,586,587

PINE, red (P. resinosa,Ait.) -- 113

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PINE, Scotch (P. sylvestris, L.) -- 122

PINE, shortleaf (P. echinata, Mill.) -- 27

PINE, slash (P. caribaea, Morelet) -- 27

PINE, sugar (P. lampertiana) -- 373,507

PINE, Virginia (P. virginiana, Mill.) -- 25,27,107

POINSETTIA (Poinsettia pulcherrima, Willd.) -- 93,214,310,339,342

Polygonum aviculare, L. -- 50,194

POPLAR, hybrid (Populas deltoides Barth. x P. trichocarpa (Torr. and Gray) -- 265,287,290

POPLAR, yellow (Liriodendron tulipifera) -- 264

POTATO (Solanum tuberosum, L.) 42,49,221,247,310,346,416

RADISH (Raphanus sativus, L.) -- 297,447,540,545,547

RYE (Secale cereale, L.) -- 477

SAFFLOWER (Carthamus tinctorius, L.)

SALVIA (Salvia splendens, Sello) -- 5

SMARTWEED (Polygonum lapathifolium) -- 22a

SNAPDRAGON (Antirrhinum majus, L.)

SORGHUM (Sorghum vulgare, Pers.)

SOYBEAN (Glycine max, Merr.) -- 34,105,171,201,205,253,271,358,450,458, 536,538,543,544,548,581

SPINACH (Spinacea oleracea, L.) -- 72,174,297,369

SPINACH (Tetragonia expansa)

SPRUCE, black (Picea glauca var. densata Bailey)

SPRUCE, blue (P. pungens, Engelm.)

INDEX OF PLANT NAMES AND REFERENCE NUMBERS[a]

SPRUCE, Norway (P. abies (L.), Karst.)

SPRUCE, white (P. glauca (Moench.) Voss.)

SQUASH, summer (Curcurbita pepo, L.) -- 297

STRAWBERRY (Fragaria, sp.) -- 463,490

SULTANA (Impatiens sultani, Hook.)

SUNFLOWER (Helianthus annus, L.) -- 411

SWEETGUM (Liquidambar styraciflua, L.) -- 563

SYCAMORE (Platanus occidentalis, L.) -- 264

TOBACCO (Nicotiana tabacum L.) -- 30,37,46,65,66,96,115-117,137,138,145, 155,198,204,222,224-226,228,237,243,247, 267,288,296,297,303,311,320,322-324,349, 353,355-357,359,360,385,424,440,443,446, 447,454,458,472,485-487,500-504,530,538, 547,548,557,575,577

TOBACCO (N. glutinosa, L.)

TOBACCO (N. rustica)

TOMATO (Lycopersicon esculentum, Mill.) -- 75,76,177,240-242,289,297,335, 349,402,418,423,454,458,498,506, 510,547,560

TOMATO (L. pimpinellifolium) -- 177

VETCH, broad bean (Vicia faba, Linn.) -- 261

VETCH, crown (Coronilla varia, L.)

WHEAT (Triticum duram, Desf.) -- 199,200,297,477,481

[a]References are in addition to those found in Table 11-24 for the same plants. For complete references on a single species, check both the Appendix and Table 11-24.

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Chapter 12

ECOSYSTEMS

The effects of ozone and other photochemical oxidants on individual species of green plants and microorganisms were discussed in detail in Chapter 11. The purpose of this chapter is to examine the effects of oxidant pollutant stress on both simple and complex communities of organisms. The human population is an integral and dependent component of these biotic communities, or ecosystems.[49]

It is usually not possible to interpret the meaning of a stable ecosystem for man's welfare in the terms of conventional economics, for example, as a cost-benefit analysis. In the case of some natural ecosystems, there may be only social and psychologic values to be considered,[78] which are only remotely related to monetary values through the recreational opportunities that they offer.[79] One of the most important justifications for the examination of the effects of oxidant stress on an ecosystem is the concern that it will not revert to its prior condition even after removal of the stress. For example, it has been suggested that the arid lands of India are the result of defoliation and elimination of vegetation, which induced local climatic changes that were not conducive to the reestablishment of the original vegetation.[7]

A natural ecosystem is a distinct association of plants and animals with the physical environment that controls them. Spatial boundaries of ecosystems are defined when the physical and biologic parts form an integrated unit in which there are defined paths for energy flow and material transport or cycling. Classes of terrestrial ecosystems called "biomes" are distinguished by their dominant vegetation form -- e.g.,

grasslands and deciduous and coniferous forests. Additional characteristics are emphasized in Odum's more formal definition: "any unit including all of the organisms (i.e., the community) in a given area interacting with the physical environment so that a flow of energy leads to a clearly defined trophic structure, biotic diversity, and material cycles (i.e., exchange of materials between living and non-living parts) within the system is an ecological system or ecosystem."[50] The agroecosystem is defined as "a unit composed of the total complex of organisms in the crop area together with the overall conditioning environment and as further modified by the various agricultural, industrial, recreational, and social activities of man."[63] The important components and processes of natural ecosystems are summarized in Table 12-1.

Ecosystems may be described developmentally as young (seral, successional) or mature (climatic). In a young ecosystem, developmental stages or communities are rapidly replaced by other communities. This succession leads ultimately to the mature stage. The distinguishing characteristic of the mature, or climax stage is that the dominant species that form the community can replace themselves; thus, the community is in equilibrium with its normal environment.

There are important differences among the components and processes of young and mature ecosystems that result in different degrees of response to environmental stresses or new perturbations, such as the presence of oxidant air pollutants (Table 12-2). The greater diversity of a mature ecosystem slows down the disruption of normal structure and function. For example, a forest ecosystem in which many species make up the producer community would show less immediate visible damage than a successional stage with only a few species. Even greater damage would be anticipated

Table 12-1

Elements of an Ecosystem

Components:

Inorganic substances (C,N,H_2O, etc.)

Organic substances

Climatic regime

Autotrophs (producers)

Phagotrophs (macroconsumers)

Saprotrophs (microconsumers)

Processes:

Energy flow circuits

Food chains (trophic relationships)

Diversity patterns in time and space

Nutrient (biogeochemical) cycles

Development and evolution

Control

Table 12-2

Characteristics of Ecosystem Development

Agroecosystems and Successional (Young) Stages	Climax (Mature) Stages
High production:respiration ratio	High biomass:respiration ratio
High net production	Low net production
Short food chains	Complex food webs
Small organisms	Great diversity in size and numbers of organisms
Open nutrient cycles	Closed nutrient cycles
Lack of stability	High stability
Maintenance cost reduced by energy subsidies (by man); production increased	Maintenance cost high, but rarely subsidized

in an agroecosystem (which might be considered the simplest of successional stages, inasmuch as often only a single producer species is present).

GENERAL RESPONSES OF NATURAL ECOSYSTEMS AND AGROECOSYSTEMS TO STRESS BY OXIDANTS

Ecosystems subjected to oxidant air pollutants must be carefully observed and described individually if we are to understand and predict the complex consequences of chronic injury. Woodwell[83] has summarized some of the expected effects of air pollutants on ecosystems: elimination of sensitive species and reduction of diversity in numbers of species; selective removal of larger overstory plants and a favoring of small plants; reduction of the standing crop of organic matter, which leads to a reduction of nutrient elements held within the living system; and increase in the activity of insect pests and in some diseases that hasten producer mortality. Many other effects can be suggested.

Photochemical oxidant air pollutants have constituted a chronic problem only during the last 20 - 25 years, at first principally in southern California, where both natural ecosystems and agroecosystems have been subjected to these pollutants for the longest period. Citrus groves and vineyards in the inland valleys of southern California are prime examples of agroecosystems stressed by chronic exposure to oxidants. Studies were initiated in 1960 on lemon and navel orange trees[72] and in 1968 on wine grapes[73] to determine the economic losses due to oxidant pollutants. Both studies were performed under field conditions for several years. The lemon and orange studies provide data that may be interpreted in an agroecosystem context, although they were highly oriented toward the primary producer components.[71, 74, 75, 76] There was no effort to examine the effects on consumer and decomposer components.

At two lemon groves and one orange grove near Ontario and Upland, California, 24 trees were selected at each grove and divided according to a randomized block design into six treatments with four replications each. The most important treatments for this discussion were greenhouse-enclosed ambient air, enclosed carbon-filtered air, and unenclosed ambient air. The first difference noted was a lower rate of water use by oxidant-stressed trees. The number of irrigations required by trees in filtered air was always significantly greater than that in ambient-air treatments; this behavior could not be correlated well with transpiration rates.[71] From a systems view, one of the major input variables, irrigation water needed, would be lower for this stressed ecosystem. Comparisons of the apparent photosynthesis of single branches of trees in filtered and ambient air yielded mixed results, but with a trend toward reduced apparent photosynthesis in ambient-air treatments.[71] After five consecutive seasons of treatment at the two lemon groves and four years at the orange grove, there were no significant differences in tree circumference among the treatments.[74] The lemon trees showed significantly greater leaf drop in both ambient-air treatments; the orange trees also followed this trend, but the differences were not significant. The drop of small, unripened fruit was a severe problem in ambient-air treatments with orange trees, but insignificant for lemons. The average annual yields at both lemon groves and the orange grove were significantly reduced when filtered versus unfiltered treatments were compared; yield was sometimes reduced by as much as 50%.[74] Additional studies with navel oranges exposed to ambient air, carbon-filtered air, and carbon-filtered air containing either ambient or half-ambient concentrations of ozone again showed increases in leaf and fruit drop and decreases in yield of marketable fruit in accordance with increasing ozone or oxidant dose.[76] None of the above studies

managed to separate the different effects of ozone and peroxyacetylnitrate or its homologues in the photochemical oxidant mixture.

In summary, oxidant stress reduced water use and photosynthesis, increased leaf drop and fruit drop, and resulted in a severe reduction in yield of marketable fruit. All these effects occurred without the development of plainly visible leaf symptoms.

Several inferences can be drawn from these data that may suggest the impacts to be expected at the consumer and decomposer levels. Accelerated leaf drop may influence the development of pests--namely, aphids, scale insects, and red citrus mites. Pest populations might be increased if injured leaves had higher concentrations of amino acids or free sugars before abscission (see Chapter 11) or diminished if leaves fall too rapidly. Leaf and fruit drop would provide a larger substrate for populations of decomposer organisms at the soil surface.

In southern California, the coastal chaparral ecosystem, dominated by chamise and manzarita or woodland species (including the live oaks and bigcone Douglas fir), and the coniferous forest ecosystem have received severe exposure; and the desert ecosystems in the vicinity of mountain passes connecting the coastal and desert regions have undoubtedly been exposed. Injury has been well documented only in the mixed-conifer forest ecosystem of the San Bernardino Mountains. Early symptoms of injury in conifer species were reported in a number of California national forests in 1970.[42] In the southern Sierra Nevada, Forest Service surveys in 1974[82] have detected increased injury in ponderosa pine since 1970 at many locations in the Sequoia National Forest, Sequoia National Park, and Kings Canyon National Park. Particular stands of mixed-conifer forest on the western slopes of the southern Sierra Nevada now appear to be affected by oxidants from the San Joaquin Valley.[44]

The potential loss of timber growth alone in this area is a very serious prospect.

Injury to important primary producer species constituting forest ecosystems is not limited to California. In the eastern United States, a disease called emergence tipburn of eastern white pine was related to ozone by Berry and Ripperton.[3] Occurrence of similar symptoms on the same species in eastern Canada could not be definitely related to ozone by Linzon.[38] The disease is characterized by bands of necrosis initiated in the semimature tissue of elongating needles; the necrosis spreads to the needle tip. In other studies with ozone fumigations at 0.07 ppm for 4 h or 0.03 ppm for 48 h, the tipburn appeared; additional symptoms were silvery or chlorotic flecks and chlorotic mottling.[13]

Under forest conditions, the affected trees occur randomly in the stand; the same trees are injured successively in a single season or in successive years.[4] Eastern white pine either forms pure stands or occurs in mixtures with other species in abandoned fields; under these conditions, it is an important pioneer tree.[80] In established stands, it is a major component of four forest types and an associate in 14 other types with a range extending over 7 million acres from the lake states to the Appalachian Mountains.[80] Berry[4] reported that emergence tipburn occurs throughout the natural range of this species; there is also evidence of a slow decline in tree vigor due to the deterioration of feeder rootlets.

Higher concentrations of ozone in the forested areas of the eastern United States would undoubtedly cause greater injury to eastern white pine and other forest species. Chapter 11 reported additional studies that suggested that other conifer species, in particular Virginia pine and jack pine, may more sensitive to ozone than eastern white pine.[16] In addition, there is a synergistic interaction between low concentrations of ozone and sulfur

dioxide that is the cause of the chlorotic dwarf disease of eastern white pine.[17] A study by Ellertsen et al.[20a] showed 10% mortality between 1956 and 1965 of dominant and codominant eastern white pines near an industrialized area including several hundred square miles on the Cumberland Plateau. Both ozone and sulfur dioxide were considered responsible for tree decline. Because eastern white pine represented only 5% of the total wood volume available for harvest, the economic impact was slight. There was no effort to interpret pollutant effects in an ecosystem context. An air monitoring network was operated by Virginia Polytechnic Institute in 1975 at three locations -- the Blue Ridge Mountains, the Shenandoah Valley, and the southern Appalachians. According to J.M. Skelly,[62] a pollution episode occurred during early July 1975 during which total oxidant peaks as high as 0.13 ppm were observed, along with 43 h when concentrations were 0.08 ppm or higher. After this episode, significant increases in oxidant injury were observed, particularly in the Blue Ridge Mountains in three categories of eastern white pine -- those previously without symptoms, those with chlorotic mottle, and those exhibiting chlorotic dwarf symptoms. Such incidences suggest the need for more comprehensive studies of oxidant (and sulfur dioxide) effects in forests of the eastern United States. In the long run, the broader question regarding effects of pollutant stress on all ecosystems components--primary producers, consumers, and decomposers--should be addressed. An analysis of the multiple effects of oxidants on eastern forest ecosystems will be a much more adequate measure of their future usefulness to man than the small amount of information now available related mainly to single primary-producer species.

The purpose of this chapter is to examine in the greatest detail possible the effects of oxidant air pollutants on ecosystems. A project is now going on to study the effects on a mixed-conifer forest ecosystem in southern California,[68,70] and the planning documents and early results from this study constitute the major source of information for the remainder of

this chapter. Other examples of damage to agroecosystems and natural ecosystems will be included.

ORIGIN OF INJURIOUS CONCENTRATIONS OF OZONE AND OXIDANTS AFFECTING NATURAL ECOSYSTEMS

Advection from Urban Centers to Remote Areas

Southern California. Descriptions of the vertical and horizontal distributions of photochemical smog in the Los Angeles basin (southern coastal air basin) during typical summer days have recently been provided by Blumenthal et al.,[5] Edinger,[20] Edinger et al.,[19] and Miller et al.[43] Important observations to be drawn from their reports are the interactions of basin and mountain topography and local meteorology in determining the transport and concentrations of oxidant air pollutants in relation to elevational zones of vegetation.

The marine temperature inversion layer that frequently forms above the heavily urbanized Los Angeles metropolitan area often extends inland as far as 90 miles (144 km), depending on season and time of day. Surface heating of air under the inversion increases with distance eastward in the basin and often disrupts the inversion by midmorning at its eastern edge. The northern rim of the basin is formed by the San Gabriel and San Bernardino Mountains, interrupted only by the Cajon Pass about 55 miles (88 km) inland (see Figure 12-1). The marine temperature inversion layer encounters the mountain slopes usually below 4,000 ft (1,200 m). In the morning, the temperature inversion often remains intact at this juncture, and air pollutants are confined beneath it. Studies by Edinger et al.[19] and Edinger[20] have described how the heated mountain slopes act to vent oxidant air pollutants over the crest of the mountains and cause the injection of pollutants into the stable inversion layer horizontally away from the slope.

Oxidant concentration within the inversion is nonuniform, containing multiple layers and strong vertical gradients. In some cases, the inversion may serve as a reservoir for oxidants, principally ozone, which may arrive at downwind locations along the mountain slopes relatively undiluted, because of a lack of vertical mixing within the inversion layer and a lack of contact with ozone-destroying material generated at the ground. The important result of the oxidant's being trapped in these layers is its prolonged contact with high terrain at night.

The most important effect of the interaction of pollutant and inversion layer at the heated mountain slope is the vertical venting of oxidants over the mountain crest by upslope flow. A gradient of oxidant concentrations or doses is established across distinct vegetation zones ordered along an elevational gradient--i.e., the chaparral and conifer forest ecosystems that occupy the slopes and mountain terrain beyond the crest, respectively. The altitudinal sequence of these ecosystems is illustrated in Figure 12-2. According to Horton,[27] the chaparral zone is subdivided from lower to higher elevations into three subzones called the chamise, manzanita, and woodland chaparral; the mixed-conifer forest occupies the mountain crest.

The daytime changes in oxidant concentrations at several stations (A, B, C, and D in Figure 12-1) along the southern slope and at the crest of the San Bernardino Mountains is illustrated in Figure 12-3. In the late afternoon, the highest concentrations in this profile were at 3,000 - 4,000 ft (900 - 1,200 m) and adjacent to the mountain slope. The oxidant was not always confined below the inversion layer, but was present in the inversion layer and above the mountain crest.[19] An instrumented aircraft measured concentrations of oxidant ranging from 0.05 to 0.11 ppm as high as 2,432 m (approximately 1,033 m above the ridge crest) during several noon and 4 p.m. flights. At these

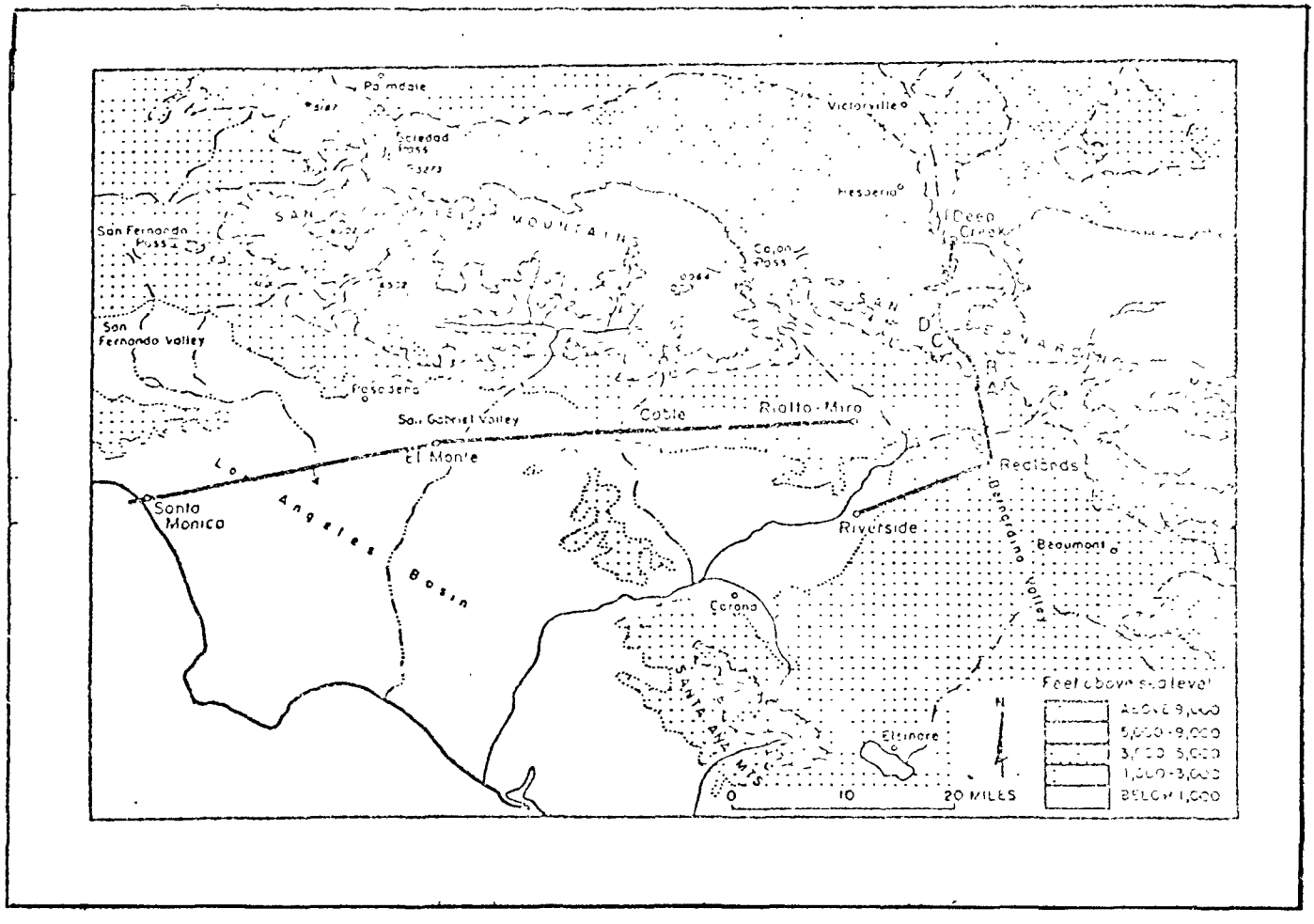

FIGURE 12-1. Major topographic features of the Los Angeles basin with inland valleys and mountains. Station locations: A, Highland; B, City Creak; C, Mud Flat; D, Rim Forest. Aircraft flight paths for the study area are also shown. (Reprinted with permission from Edinger et al.[19])

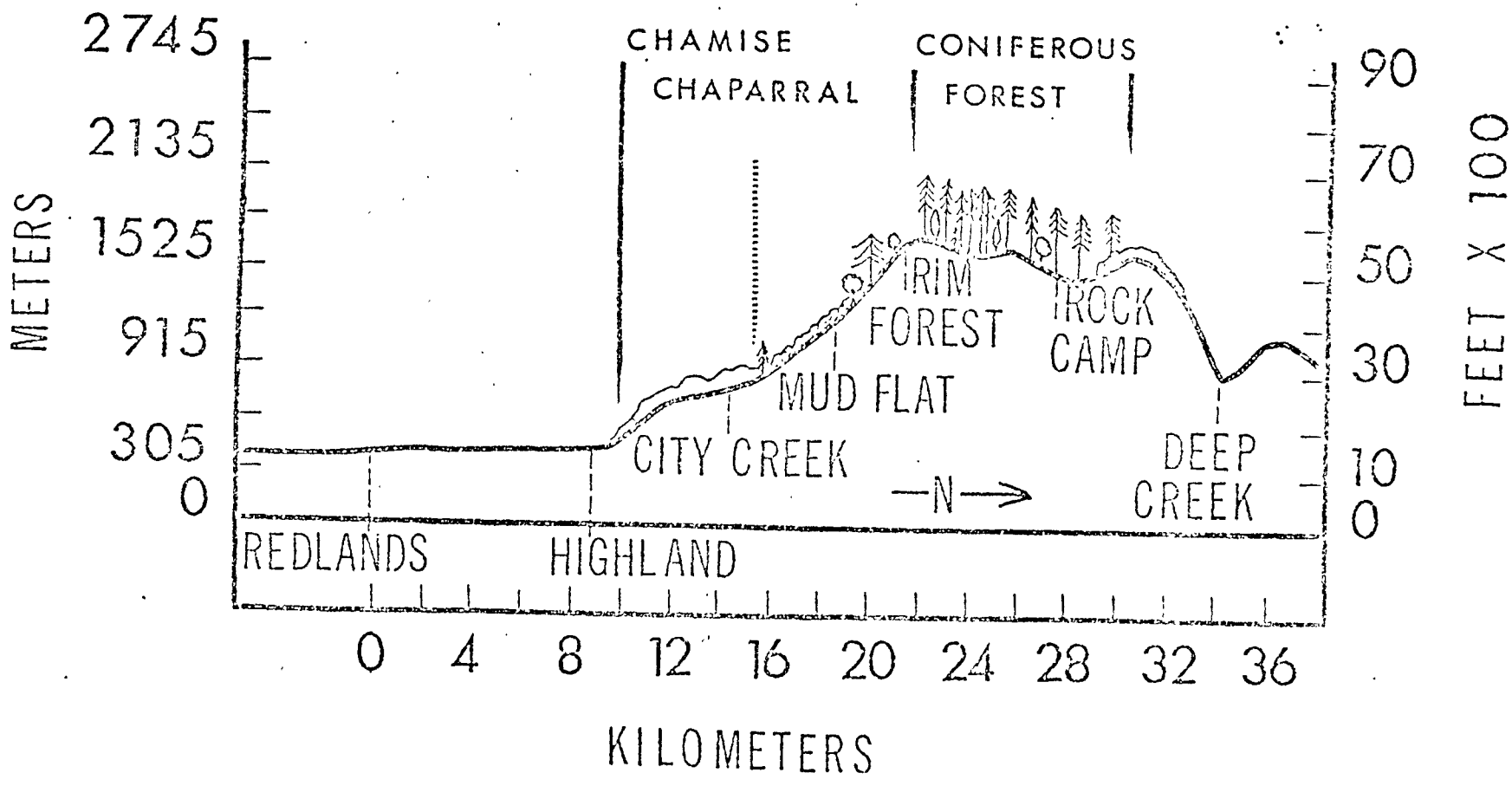

FIGURE 12-2. Altitudinal sequence of ecosystems in the San Bernardino Mountains. (Reprinted with permission from Miller et al.[43]

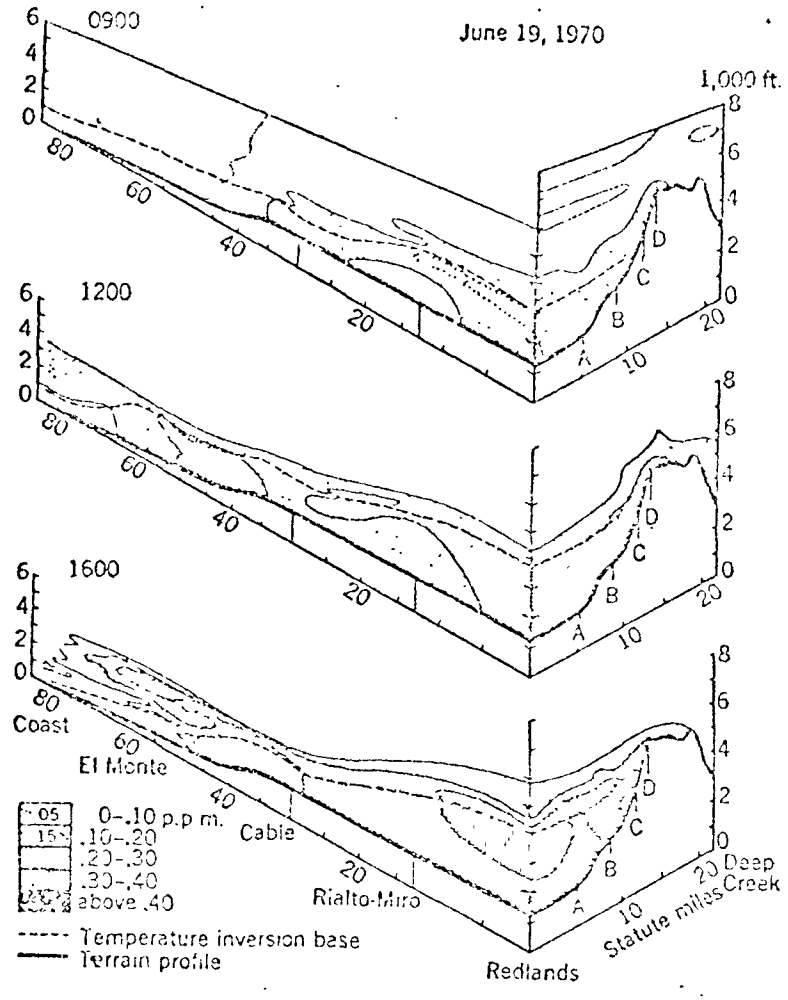

FIGURE 12-3. Daytime changes in oxidant concentrations along a west-to-east transect in the southern coastal air basin, including the slopes of the San Bernardino Mountains (see Figure 12-1). (Reprinted with permission from Edinger et al.[19])

times, downwind diffusion of oxidant beyond the crest--on the basis of aircraft flights approximately 500 ft (150 m) above the terrain--suggested only slight dilution in the first 10 km north of the crest.[19] Observations on other days[43] suggested a 50-60% reduction in oxidant 10 km north of the crest. There was no ground station at this point for comparison with the aircraft measurements at either time.

Total oxidant, temperature, and vapor-pressure gradient were measured continuously during 16 days in July and August at mountain slope and crest stations (A, B, C, and D in Figure 12-1). In Figure 12-4, the time of the daily peak oxidant concentration was progressively later at stations of higher elevation. Temperatures and vapor-pressure gradients were also progressively lower at higher elevations at the time of oxidant peak. The duration of oxidant concentrations exceeding 0.10 ppm was 9, 13, 9, and 8 h/day going from lower to higher stations. The longer duration at City Creek (elevation, 817 m) probably coincides with the point where the inversion layer most often contacts the mountain slope. The oxidant concentrations rarely decreased below 0.05 ppm at night on the slope of the mountain crest, whereas it usually decayed to near zero at the basin station (Highland).

The vegetation zones along the slope and at the crest are subjected to oxidant exposure differently. Even though the lower chaparral receives longer exposure, the peak concentrations coincide more closely with the maximal evaporative stress for the day. There is some support for the hypothesis that stomates would be closed during this period and that pollutant uptake would be lower. There is in fact very little visible injury to the species in this zone. In contrast, the daily oxidant peak occurs well after the temperature and vapor-pressure gradient maximums in the conifer forest at the mountain crest; the oxidant injury to plant species here is severe. These suggestions of strong microclimatic control of the sensitivity of these native species to ozone form a working hypothesis that needs further investigation.

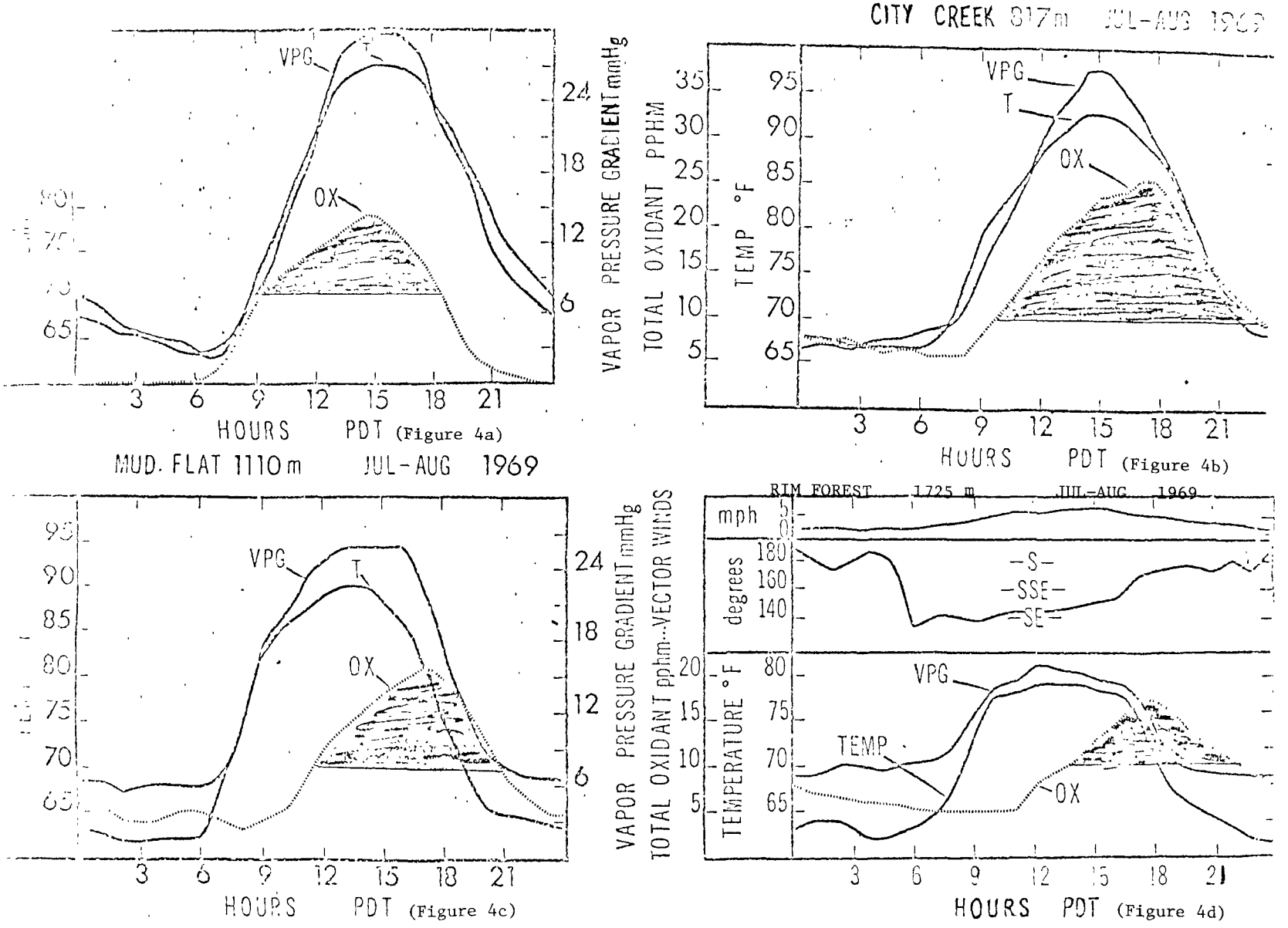

FIGURE 12-4. The relationship of times of occurrence of the daily oxidant peak concentration to temperatures and vapor-pressure gradients in an elevational sequence (see Figures 12-1 and 12-2) on the slopes of the San Bernardino Mountains. (Reprinted with permission from Miller et al.[43])

The San Joaquin Valley and Adjacent Sierra Nevada Mountains.[42,82] Field surveys have confirmed oxidant injury to ponderosa pine and associated species at numerous locations in the Sierra Nevada foothills east and southeast of Fresno. Oxidant measurements at ground stations and by instrumented aircraft show late-afternoon peaks of "transported" oxidant on the western slopes of the Sierras. Limited measurements by instrumented aircraft suggest the development of a layer of oxidant approaching the forested mountain slopes between 610 and 1,829 m during the late afternoon.[44] A very weak inversion or isothermal layer may serve as a reservoir of oxidant, which is advected to the mountain slope in the southern coastal air basin, as suggested by Edinger.[20]

Considerable concern has been registered about air quality in the Lake Tahoe Basin, where local development may cause adverse oxidant concentrations.[9]

Seasonal and Daily Variations of Injurious Concentrations

Synoptic Weather Patterns Associated with Episodes of High Pollution. McCutchan and Schroeder[40] classified 145 days during May through September 1970 with data collected on the southern slopes of the San Bernardino Mountains. Five general weather patterns were described, with the associated synoptic patterns at the surface and at 500 millibars (Table 12-3). An analysis of eight meteorologic variables was used to classify days during May through September into the five general weather categories. Of 123 days classified by stepwise discriminant analysis, 10, 13, 44, 23, and 24 were correctly categorized into classes 1-5, respectively; the remainder could not be clearly placed in the five classes. The oxidant concentrations associated with these classes were: 1, low; 2, low and high; 3, high; 4, low; and 5, low. In this sample, the sum of days in classes 2 and 3, namely 57 days, suggests that about 46% of the sample days had high concentrations of oxidant air pollutants.

Table 12-3

Descriptions of Meteorologic Patterns for Five Classes of Spring and Summer Days in Southern California[a]

Class	General Weather	Associated Synoptic Pattern at Surface	Associated Synoptic Pattern at 500 mb
1	Hot, dry continental air throughout the day (Santa Ana)	Large high pressure over Great Basin	Strong northerly winds over area with trough east of the area
2	Relatively dry forenoon; modified marine air in afternoon; very hot (heat wave)	High pressure over Great Basin and thermal trough over desert	Subtropical closed high over area
3	Moist, modified marine air; hot in afternoon	Thermal trough over desert	Ridge over area
4	Moist, modified marine air; warm in afternoon	Thermal trough over desert	Trough over area
5	Cool, moist, deep marine air throughout the day	Synoptic low over desert	Deep trough or closed low over area

[a] Derived from McCutchan and Schroeder.[40]

Class 2 and 3 days are both characterized by high pressure (500-mb ridge over the area). Descriptions of the synoptic weather patterns (contained in the U.S. Forest Service "California Fire Weather Severity, 10-Day Summaries") for May through August 1972-1974 were compared with episodes of severe oxidant concentration at Rim Forest and nearby Sky Forest. For the purposes of this comparison, all days having maximal hourly concentrations of 0.33 ppm or higher were compared with the synoptic patterns for those days. In all cases, a persistent 500-mb high pressure over the area was the most common synoptic feature. For 8 qualifying days in 1972, the mean of the maximal hourly concentrations for those days was 0.37 ppm; for 16 days in 1973, 0.41 ppm; and for 46 days in 1974, 0.38 ppm. The highest hourly concentration ever obtained was 0.60 ppm, on June 28, 1974.

During episodes of severe oxidant pollution, the weather is generally very hot (85-100 F or about 29-38 C) and the relative humidity may be either low or moderately high on class 2 and 3 days, depending on the behavior of the marine layer. The small difference between means of maximal hourly concentrations on high-pollution days in 1972-1974 suggests continuation of heavy primary pollutant emission in spite of current control strategies.

Annual Trends of Total Oxidant Concentrations at a San Bernardino Mountain Station and the Nearby City of San Bernardino. Since 1968, total oxidant concentrations have been measured continously with a Mast ozone meter (calibrated by the California Air Resources Board method) from May through September at Rim Forest-Sky Forest.[31a] The fall, winter, and early spring months have generally been omitted until recently, because synoptic patterns are usually not conducive to oxidant accumulation and transport. For example, average maximal hourly oxidant concentrations from October through April 1973 and 1974 stayed below 0.10 ppm; those for April were 0.10-0.15 ppm.[70] The

main data collection period coincides with the growing season and thus permits a reasonable estimate of the total annual dose of oxidant air pollution received by vegetation.

Two methods of documenting trends of oxidant concentrations during the 1968-1974 period at Rim Forest-Sky Forest express the accumulated dose as a sum of all hourly values in micrograms per cubic meter and as number of hours with a concentration of 0.08 ppm or more (the federal standard) for each month separately, June through September (Figure 12-5). The dose excludes background concentrations -- those less than 59 $\mu g/m^3$ (0.03 ppm). The percentage of valid data recovered is also indicated. The absence of some data -- ranging up to 17.8% in 1970, but averaging 8.3% during the 7 years -- represents an underestimate that cannot be adjusted with any certainty. The total number of hours with concentrations of 157 $\mu g/m^3$ (0.08 ppm) or more during June through September was never less than 1,300 during each of the 7 years. These measures of oxidant trends indicate no improvement in air quality.

Data are available back to 1963 from the downtown San Bernardino station operated by the County Air Pollution Control District (APCD). The colorimetric potassium iodide method used to measure total oxidants was calibrated according to the method of the California Air Resources Board. A positive correction factor of 1.22 was used to adjust mountain data for the decreased air pressure at the higher elevation.

The data from the Rim Forest-Sky Forest station were compared with published data from the San Bernardino County APCD. The numbers of hours with concentrations exceeding 392 $\mu g/m^3$ (0.20 ppm) during July, August, and September in 1969 - 1974 were compared (Figure 12-6). For 1963 - 1968, data are shown only from the San Bernardino APCD. A large part of the year-

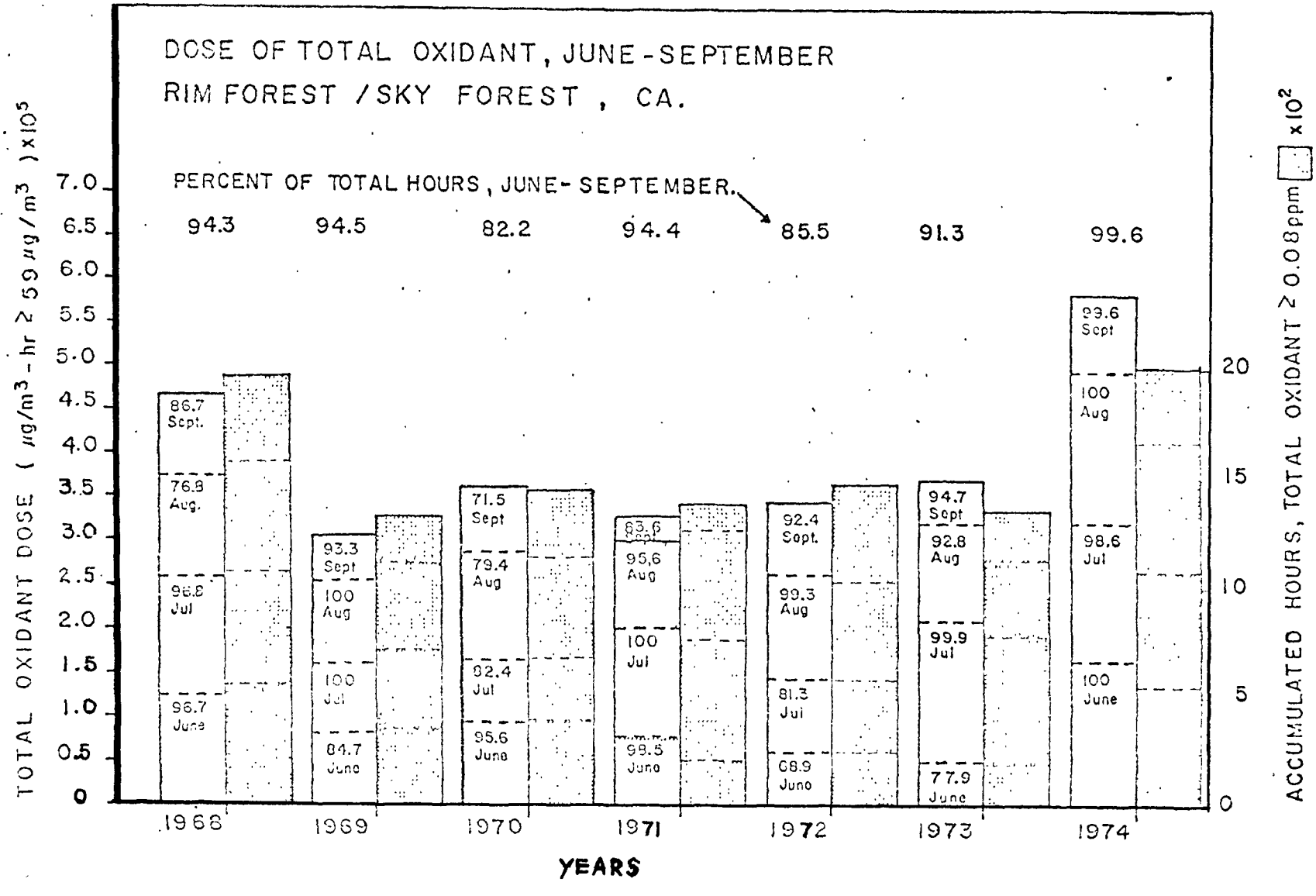

FIGURE 12-5. Monthly summation, June-September 1968-1974, of total oxidant dose and total number of hours at a dose equal to or greater than 157 µg/m^3 (0.08 ppm) at Rim Forest-Sky Forest in the San Bernardino Mountains. Numbers in open bars indicate percentage of total possible hours for which data were available. (Reprinted with permission from Kickert et al.[31a])

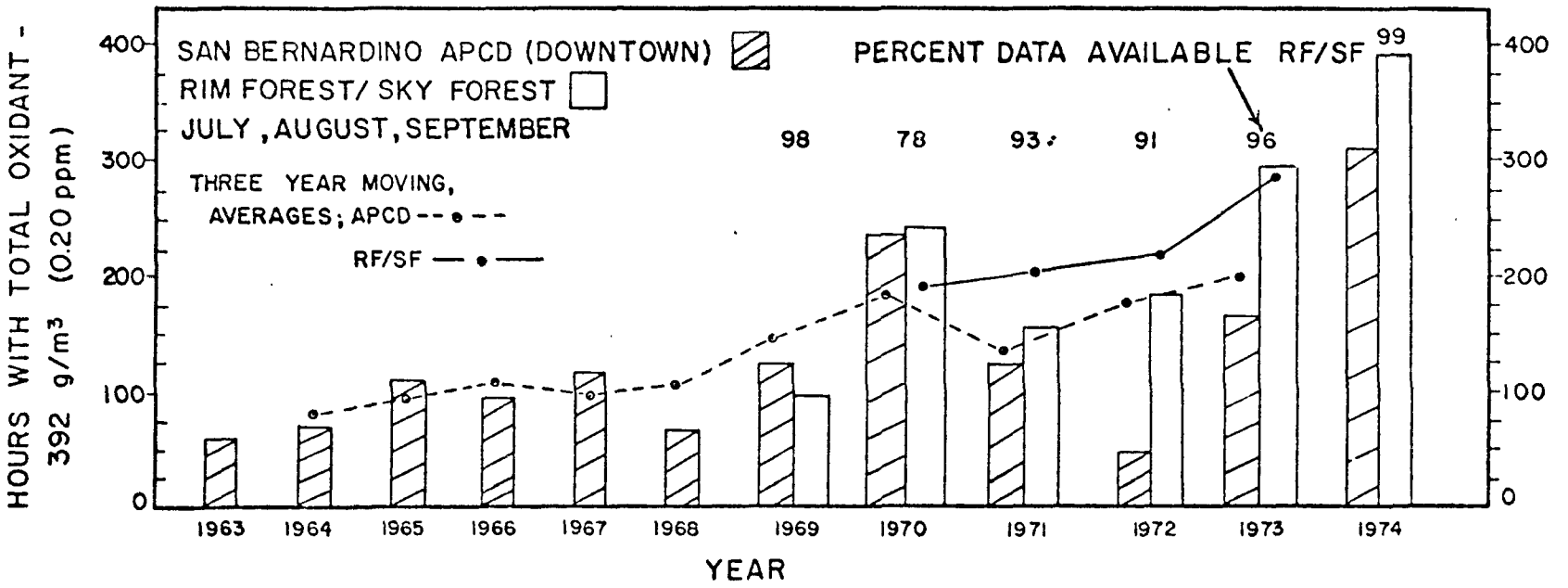

FIGURE 12-6. Number of hours of total oxidant, July-September, equal to or greater than 392 $\mu g/m^3$/0.20 ppm, at downtown San Bernardino Country on Pollution Control District Station, 1963-1964, and Rim Forest-Sky Forest, 1968-1974. (Reprinted with permission from Kickert et al.[31a])

to-year differences at the same station and between stations can be attributed to differences in synoptic and mesoscale (local) meteorologic patterns. For example, the increases in 1972, 1973, and 1974 at Rim Forest-Sky Forest are associated with 6, 16, and 46 days, respectively, when a persistent 500-mb ridge occurred over the Southwest, particularly southern California. The difference between stations in the same year is probably influenced most by inversion height. Lower inversions partially restrain transport upslope to shorter periods each day. Higher inversions would have the opposite effect and also allow a greater air volume below to dilute oxidants. The index for comparison chosen in Figure 12-6 -- i.e., hours $\geq$ 392 $\mu g/m^3$ would be sensitive to inversion height. The 3-year moving averages for each station tend to remove some of the variation due to meteorology. In terms of hours equal to or exceeding 392 $\mu g/m^3$, the moving average between 1970 and 1973 at Sky Forest-Rim Forest has increased from 175 to 290. The increased oxidant concentrations at both these stations are the reverse of those in upwind, urban Los Angeles County, where increased emission of NO_x tends to shift the chemical equilibrium to the left, toward the ozone precursors. The most recent data[11] firmly indicate that oxidant concentrations will either increase annually or continue to oscillate around the mean of present high concentrations in the foreseeable future at these distant locations.

The tropospheric ozone cycle[11] describing rural upwind, urban, and rural downwind variations in concentration can be easily demonstrated in the Los Angeles and connected inland basins. The effects on both natural ecosystems and agroecosystems also become apparent. For example, oxidant doses during August 1972 at six oxidant stations -- Costa Mesa, La Habra, Corona, Riverside, City Creek, and Rim Forest -- extending in a line

northeastward from the coast to the mountains show low doses at the coast increasing to a maximum on the chaparral-covered mountains and decreasing beyond the mountain crest (Figure 12-7). The oxidant dose is indicated by the dashed line. A crude estimate of the relative economic value of the products from ecosystems encountered along this transect are expressed in a very general way by the solid line and the nomenclature on the abscissa. Finally, the relative complexity of the ecosystems involved is shown below the abscissa. This conceptualization emphasizes the enormously greater dosage received by the natural ecosystems during this month. This pattern of dosages is very typical of June, July, and August, but the offshore flow typified by the Santa Ana winds may reverse the situation in September or October; susceptible crops growing on the coastal plain may be seriously damaged.

In general, the permanent vegetation constituting natural ecosystems receives much greater chronic exposure, whereas the short-lived higher-value vegetation constituting the agroecosystems of the coastal plain can be subject to injurious doses, but in intermittent short-term fumigations. Each situation has measurable economic and aesthetic effects, but on different time scales. The simple agroecosystem has little resiliency to pollutant stress; losses are immediate and may be catastrophic. The complex natural ecosystem is initially more resistant to pollutant stress, but the longer chronic exposures cause the disruption of both structure and function in the system. Damage may not be reversible.

AN EXPERIMENTAL DESIGN FOR INVESTIGATING THE EFFECTS OF OXIDANT STRESS ON ECOSYSTEMS

Techniques for Ecosystem Modeling

The steps needed to understand the effects of oxidant air pollutants on an ecosystem are description of ecosystem components or subsystems

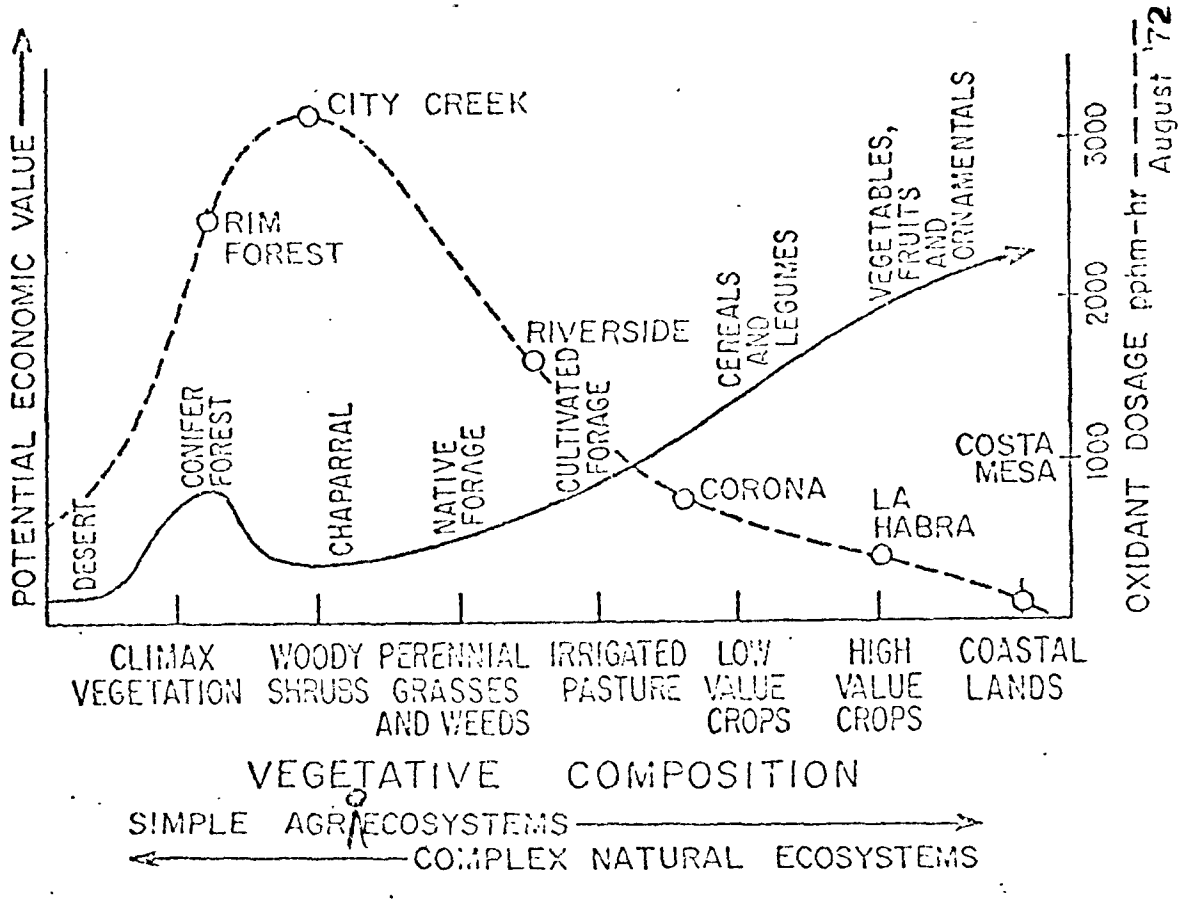

FIGURE 12-7. A hypothetical expression of the relationships of oxidant dose, natural ecosystems, agroecosystems, and the relative values of their vegetative components along a transect in the southern coastal air basin of California. The only real data are oxidant doses, which define the pollutant concentration gradient from the coast to the interior.

and identification of the most important processes within organisms or interactions between organisms or communities of organisms that would be most vulnerable to pollutant stress, including an evaluation of factors of the physical environment that may limit ecosystem processes. This information can be conveniently arrayed in terms of interaction tables that show which variables are involved in various subsystems and whether the interaction between any two variables is positive or negative. The next step involves the selection of the most appropriate paradigm for the substance or "unifying thread" that flows through the various subsystems. Examples of possible "flows" are energy, biomass, mineral nutrients, water, numbers of species, population densities, or area occupied per biotic unit.

The state of the art of ecosystem modeling has evolved rapidly in recent years. Discussions of various ecologic subsystem simulators are published.[30, 34, 52] Recently, discussions of the utility of computer simulation modeling in ecologic research have been published by the National Academy of Sciences[47] and the National Science Foundation.[48]

Depending on the "flows" through the ecosystem that one chooses to study, the modeling procedures may be either deterministic or stochastic. Rochow[58] has described the step-by-step procedure for studying the flow of nitrogen through a forest ecosystem. In this case, nitrogen can be found in a number of well-defined storage compartments. The transfers of nitrogen between compartments are described by transfer coefficients arrayed in a symmetric transfer matrix. Differential equations are written that represent the rate of change (or flow) of nitrogen through time for each of the various compartments. Computer programs already exist to solve these equations. Once the model is running smoothly on the computer, it is possible to impose

various experimental conditions -- e.g., clear-cutting or selective cutting -- and observe the effects on nitrogen flow. Sensitivity analysis can then be performed to study which transfer coefficients are sensitive to small changes and which only to large changes. The useful outputs of these manipulations include a determination of how much particular transfers in the system can be changed or stressed before the ecosystem deteriorates. This example uses a deterministic model; however, the flow of water through an ecosystem may have both deterministic and stochastic mathematical properties.[15]

Models of energy flow or biomass flow have been more successful when uniform plant canopies were involved -- e.g., a field of corn. A recent development from the field of integrated pest management has been described in which an alfalfa growth model has been coupled with an alfalfa weevil population dynamics model.[23] The plant growth model in this example might be adaptable to air pollutant injury studies in agroecosystems. Because the model is built to incorporate current weather data that have direct influences on pollutant concentration and plant sensitivity, this type of model seems to be a worthwhile departure point for simulating dose responses.

The simulation of the population structure and dynamics of autotrophs and phagotrophs is another important interaction that can be modeled to test for effects of pollutant stress. A standard approach is the use of a finite-population-difference model.[35] The model assumes that the population change of a species in a specific period is equal to the species population multiplied by an intrinsic coefficient of rate of change. The rate coefficients are difficult to define without extensive data. The task is further complicated because "a consistent feature of communities is that they contain a comparatively few species that are common-- that is, represented by large numbers of individuals or a large biomass--and a comparatively large number

of species that are rare at any given locus in time and space."[49] It is implied by this statement that adequate sampling procedures may be difficult to design if only the spatial aspect of the problem is considered. The constraints of short-term research funding in the form of grants or contracts generally preclude adequate sampling of the time dimension. Ewing et al.[21] have considered a related modeling constraint: the simulation of the hierarchic structure inherent in a biologic population. Through exploitation of the structure of the biologic, mathematical, and computer systems, a Monte Carlo simulation of a structured population is possible. With a nonhomogeneous Poisson process, events are defined and a suitable risk structure is developed that satisfies mathematical and biologic requirements. A structured computer language allows one to mimic the structure and organization of a population -- different levels of organization ranging from the individual to the complete population.

A description of another "flow," the area occupied per biotic unit over time, is also very important for defining and predicting long-term successional changes in plant communities due to pollutant stress. Schugart et al.[61] have described a model for 250 years of succession in the western Great Lakes Region in the absence of fire, epidemics, and forest management. Botkin and Miller[6] predicted successional changes during a 100-year period after logging in a northern hardwood forest.

There are no examples of models dealing directly with oxidant stress on communities or ecosystems. However, the types of models just described may be applicable to this problem. In view of the trend of increasing oxidant concentrations downwind from urban areas,[5] a much more precise understanding of chronic effects on both natural ecosystems and agroecosystems is required. Descriptive and predictive models dealing with the responses of biologic systems can provide the best input to the

decision models needed for standard-setting, land-use planning, and resource management.

Modeling the Effects of Oxidant Stress on a Western Mixed-Conifer Forest Ecosystem

The recent history of the mixed-conifer forest of the San Bernardino Mountains has been analyzed.[68] This analysis included an initial inventory of ecosystem components and processes, as indicated in Figures 12-8 through 12-12. The inventory emphasizes ponderosa and Jeffrey pines, the most dominant species in the climax community, and is stratified according to organizational level -- namely, organism or tree (Figures 12-8 and 12-9), community or stand (Figures 12-10 and 12-11), and finally a time-oriented analysis of plant succession in the aggregation of relatively distinct communities or stands that collectively make up the mixed-conifer forest ecosystem (Figure 12-12). This analysis was assembled by nearly a dozen coinvestigators representing many disciplines.[70] These detailed diagrams are presented here to emphasize the complexity of ecosystem interactions at each level of biologic organization. Only the interactions considered to be the most important ones guiding the course of plant succession in the several forest plant communities now constituting the mixed-conifer forest have been selected by project investigators for immediate investigation.[69] In the case of each interaction, it is important to decide the time frequency at which the selected state variables and driving variables will be measured--i.e., hourly, weekly, or annually.

A data capture, storage, and retrieval system is being devised to provide modelers with immediate access to all subproject data. Because this project is currently the most active effort directed toward understanding the effects of ozone and other oxidants on a natural ecosystem, most of this chapter is a discussion of its latest progress.

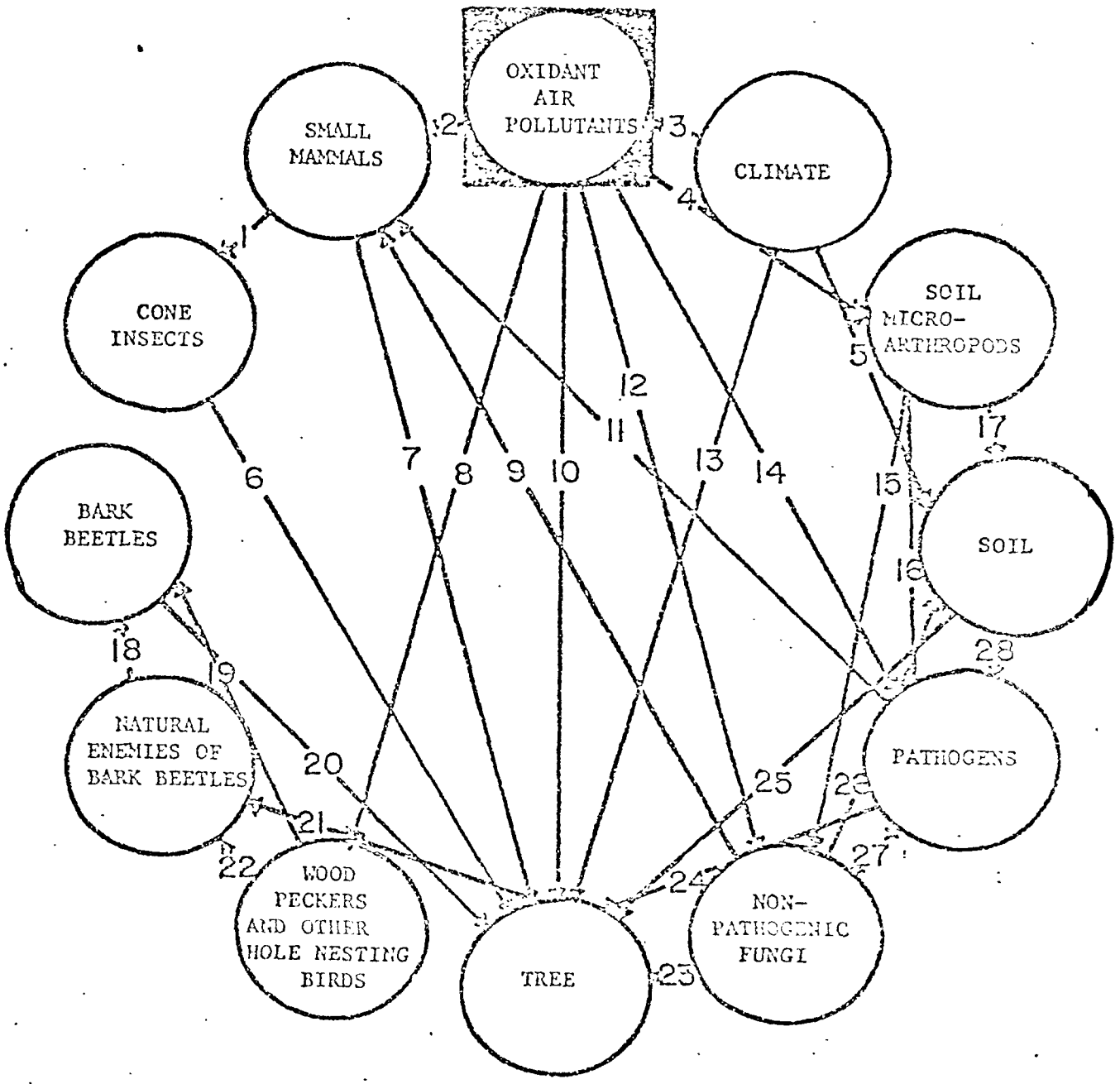

FIGURE 12-8. Organism-level interactions in a mixed-conifer forest. Types of interaction: 1, competition for food supply; 2, direct effect of ozone on small-mammal physiology; 3, climatic control of oxidant concentration in forest; 4, direct effect of ozone on soil micro-arthropods; 5, effect of precipitation and temperature on soil moisture and soil temperature; 6, insect damage to developing cones; 7, small-mammal damage to developing cones and importance of cone crop as animal food supply; 8, direct effect of ozone on physiology of cavity-nesting birds; 9, role of fruiting bodies of nonpathogenic fungi in nutrition of small mammals; 10, direct effect of ozone on tree physiology; 11, role of fruiting bodies

of pathogens in nutrition of small mammals; 12, direct effect of ozone on nonpathogenic fungi; 13, effect of temperature and evaporative stress on tree growth; 14, direct effect of ozone on pathogenic fungi; 15, interaction of nonpathogenic fungi and soil microarthropods; 16, interaction of pathogenic fungi and soil microarthropods; 17, effect of soil microarthropods on litter reduction; 18, impact of predation and parasitism on bark beetles; 19, predation of bark beetles by woodpeckers; 20, effect of bark beetles on tree mortality and vigor and effect of phloem thickness and moisture on bark beetles; 21, effect of phloem moisture and thickness on natural enemies of bark beetles; 22, influence of woodpeckers on rate of parasitism; 23, effect of nonpathogenic fungi on tree growth; 24, effect of pathogens on tree vigor and mortality; 25, effect of soil moisture and soil temperature on tree growth; 26, effect of soil moisture and temperature on occurrence of nonpathogenic fungi; 27, interaction of pathogenic and nonpathogenic fungi; 28, effect of soil moisture and temperature on occurrence of pathogenic fungi. (Reprinted with permission from Taylor.[70])

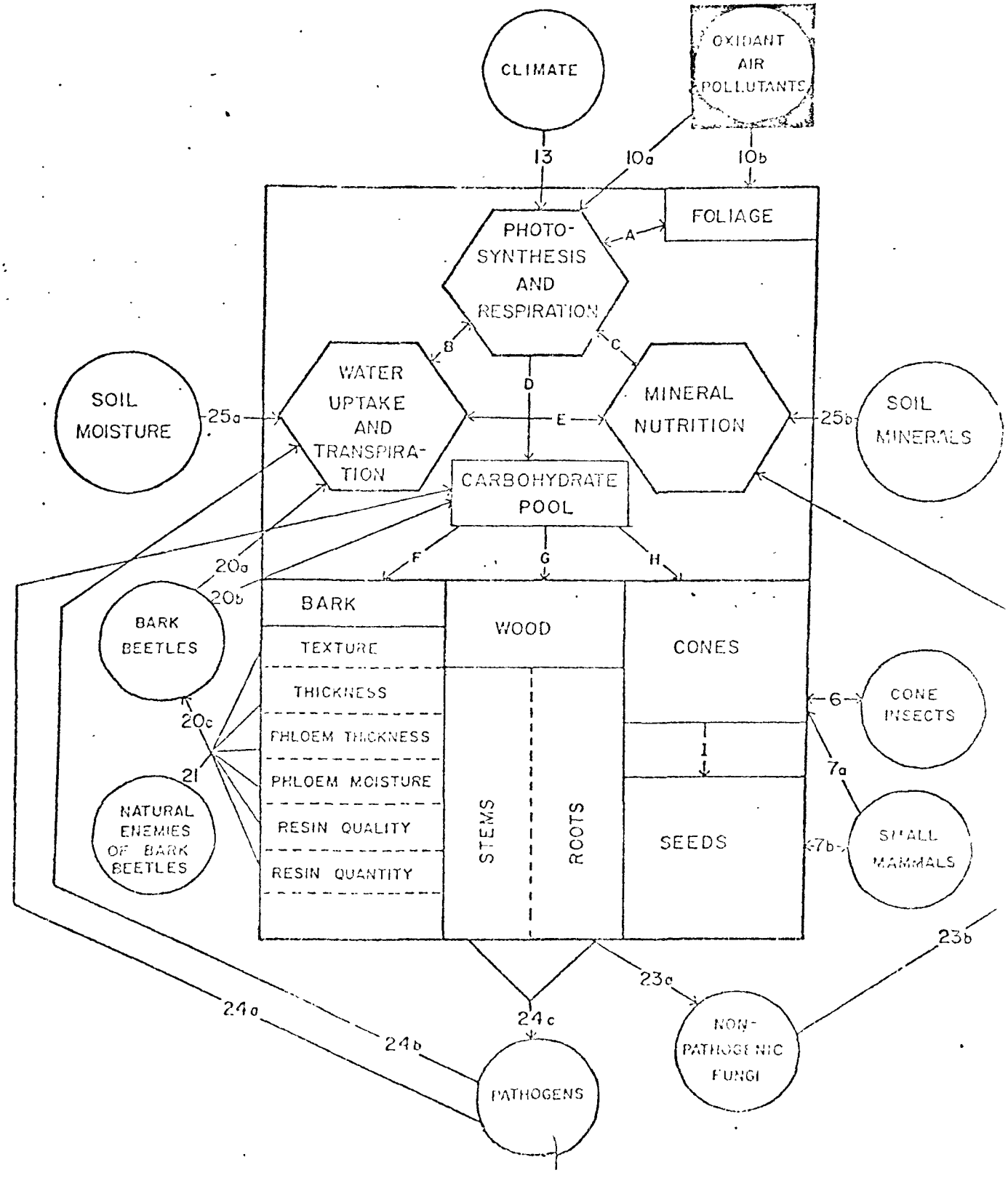

FIGURE 12-9. Tree-level interactions in a mixed-conifer forest ecosystem. Types of interaction: A, relationship between net photosynthesis, foliage age, and foliage retention; B, effect of plant moisture stress on net photosynthesis; C, relationship between mineral

uptake and net photosynthesis; D, relationship between net photosynthesis and carbohydrate storage; E, relationship between water uptake and mineral nutrition; F, relationship between carbohydrate storage and bark characteristics; G, relationship between carbohydrate storage and cone production; H, relationship between carbohydrate storage and cone production; 1, relationship between cone crop and seed crop; 6, cone insect damage to developing cones; 7a, small-mammal damage to developing cones; 7b, small-mammal predation of seeds; 10a, effect of ozone on photosynthesis and respiration; 10b, effect of ozone on needle retention; 13, effect of temperature, light intensity, and evaporative stress on photosynthesis and respiration; 20a, effect of bark beetles on water uptake and transpiration and relationships of tree moisture stress to bark beetle attack; 20b, effect of bark beetles on tree carbohydrate concentration and relationships of carbohydrate concentration to bark beetle population in tree; 20c, relationships between bark characteristics and bark beetle attack and population; 21, relationship between bark characteristics and natural enemies of bark beetles; 23a, relationship between root characteristics and mycorrhizal-forming nonpathogenic fungi; 23b, effect of nonpathogenic fungi on mineral uptake; 24a, effect of pathogens on tree carbohydrate concentration and relationship of carbohydrate concentrations to pathogen attack; 24b, effect of pathogens on water uptake and transpiration; 24c, relationship between stem and root characteristics and pathogen attack; 25a, effect of soil moisture and temperature on water uptake and transpiration; 25b, effect of soil mineral concentration and temperature on mineral nutrition of tree. a indicates direct influence of external factor on trees. b and c indicate influence of tree condition on external factor. (Reprinted with permission from Taylor.[70])

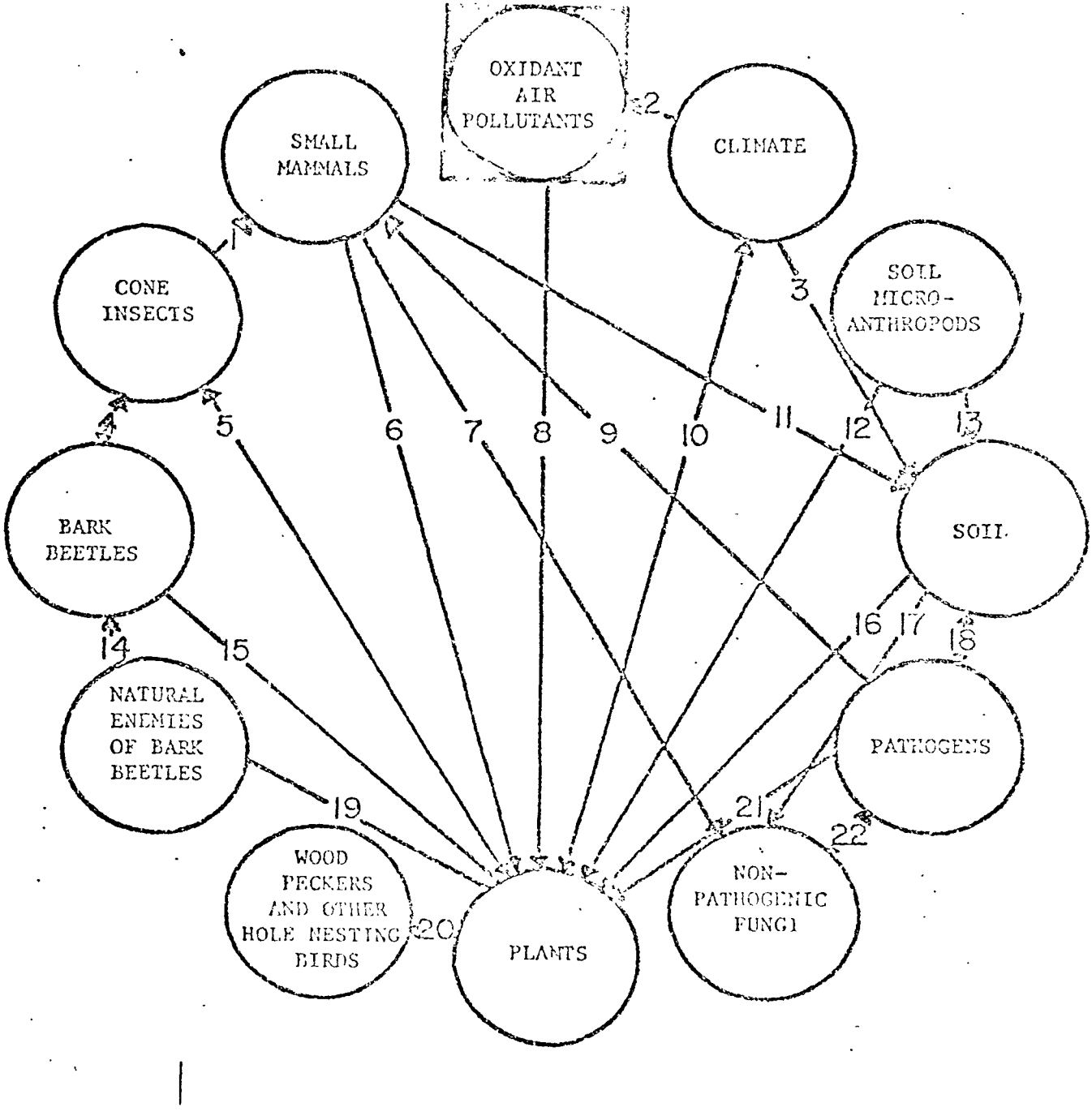

FIGURE 12-10. Community-level interactions in a mixed-conifer forest ecosystem. Types of interaction: 1, competition between woodpeckers and small mammals; 2, climate control of oxidant concentration in different forest communities; 3, effect of precipitation and

temperature on soil moisture and soil temperature in different forest communities; 4, predation of bark beetles by woodpeckers in different forest communities; 5, effect of cone crop abundance on cone insect populations in different forest communities; 6, effect of cone crop abundance on small-mammal populations in different forest communities; 7, fruiting bodies of nonpathogenic fungi as food for small mammals in different forest communities; 8, smog-caused mortality and morbity in different forest communities; 9, fruiting bodies of pathogens as food for small mammals in different forest communities; 10, effect of temperature and evaporative stress on species composition in different forest communities; 11, relationship between soil characteristics and population density of burrowing small mammals in different communities; 13, relationship between soil characteristics and microarthropod population; 14, bark beetle mortality caused by natural enemies in different forest communities; 15, effect of bark beetles on tree mortality and vigor in different forest communities; 16, relationship between soil characteristics and forest community composition and growth; 17, relationship between soil characteristics and species distribution and behavior of nonpathogenic fungi; 18, relationship between soil characteristics and species distribution and behavior of pathogens; 19, influence of forest community type on populations of natural enemies of bark beetles; 20, woodpecker distribution and density in different forest communities; 21, effect of pathogens on tree vigor and mortality in different forest communities; 22, relationship between nonpathogenic fungi and forest community composition and growth. (Reprinted with permission from Taylor.[70])

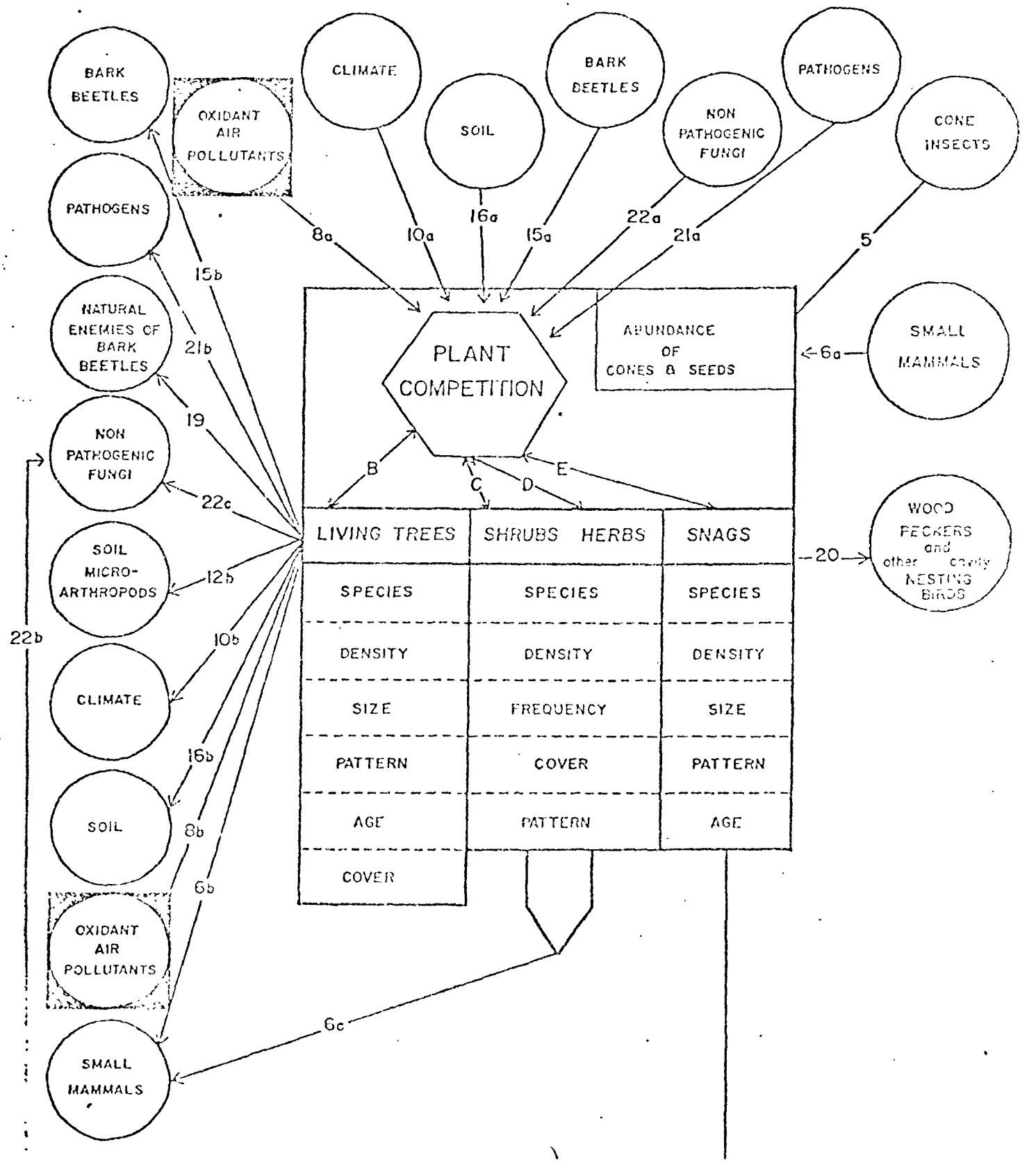

FIGURE 12-11. Stand-level interactions in a mixed-conifer forest ecosystem. Types of interaction: A, effect of plant competition on abundance of cone and seeds; B, effect of plant competition

on characteristics of living trees; C, effect of plant competition on characteristics of shrubs; D, effect of plant competition on characteristics of herbs; E, effect of plant competition on tree mortality; 5, abundance of cones and seeds and predation by cone insects; 6a, abundance of cones and small-mammal predation of cones; 6b, characteristics of forest stands and small-mammal populations; 6c, influence of shrub and herb layer vegetation on small-mammal populations; 8a, smog-caused mortality and morbidity in different tree species with forest stands; 8b, influence of stand conditions on concentration of oxidants; 10a, influence of temperature and evaporative stress on stand structure and composition; 10b, influence of stand condition on temperature and evaporative stress; 12b, influence of stand condition on soil microarthropod population; 15a, influence of bark-beetle-caused tree mortality on stand condition; 15b, influence of stand condition on bark beetle population; 16a, influence of soil moisture and temperature on stand characteristics; 16b, influence of stand condition on soil characteristics; 19, influence of stand condition on population dynamics of natural enemies of bark beetles; 20, relationship between smog occurrence and woodpecker population; 21a, influence of pathogen-caused mortality on stand condition; 21b, influence of stand condition on pathogen population; 22a, influence of mycorrhiza fungi; 22b, influence of snags (and downed trees) on nonpathogenic fungi; 22c, influence of stand condition on nonpathogenic fungi. a indicates direct influence of external factor on trees in stands. b and c indicate influence of stand condition on nonpathogenic fungi. (Reprinted with permission from Taylor.[70])

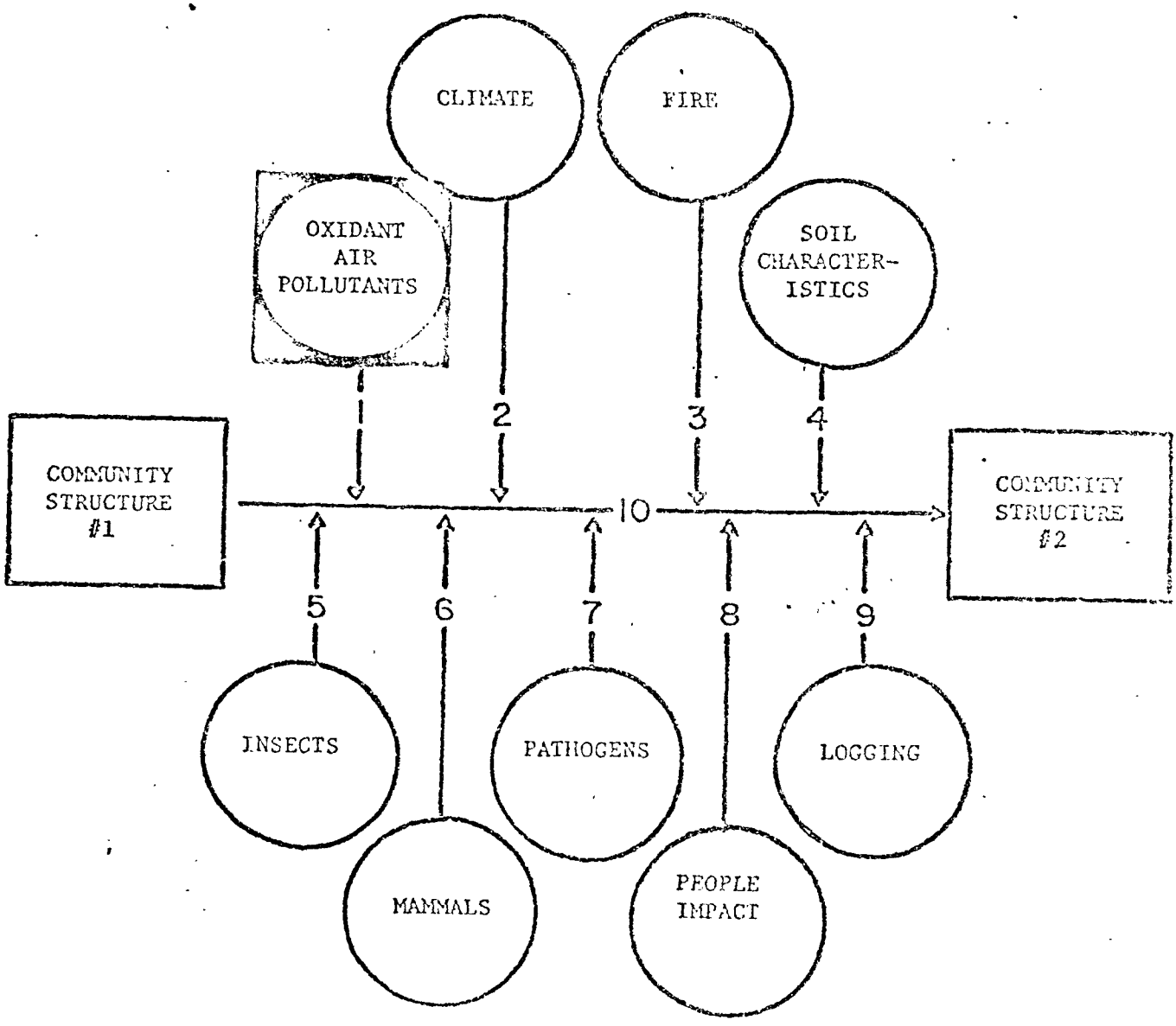

FIGURE 12-12. Community-succession interactions in a mixed-conifer forest ecosystem. Types of interaction: 1, oxidant modification of community structure; 2, climate modification of community structure; 3, fire modification of community structure; 4, soil characteristics and their influence on community structure; 5, insect modification of community structure; 6, mammal modification of community structure; 7, pathogen modification of community structure; 8, people impact on community structure; 9, logging modification of community structure; 10, predictive capability through integration of submodels for items 1-9. (Reprinted with permission from Taylor.[70])

Other researchers at the University of Utah[25, 56, 77] have completed studies to determine the ozone susceptibility of the most prevalent species in some major vegetation associations in the intermountain area. These data are intended to be combined with background information on community stability and integrated into mathematical equations. The equations may be able to predict the community changes that occur after stress of various degrees. The ozone concentrations in grassland and aspen plant communities in the Wasatch Mountains above Salt Lake City are much lower than those in the San Bernardino Mountains. According to Treshow and Stewart,[77] "Background ozone concentrations in the grassland communities geographically contiguous to the Salt Lake Valley occasionally reached peaks at 15 pphm, but the average concentration over a 1-hour period never exceeded 6 pphm at any of the plots. Concentrations measured in the aspen community during the study reached 9 pphm but never for more than a few minutes at a time." The present low concentrations may be important to some species or cultivars[25, 56] and the plant communities of which they are members.

EFFECTS ON PRIMARY PRODUCERS (GREEN PLANTS)

The ozone dose responses and the specific effects on the photosynthetic activity of both herbaceous and woody plants, principally in controlled short exposures, are discussed in Chapter 11. The main aim of this section is to evaluate the effects of the chronic exposure of vegetation in natural ecosystems to total oxidants (more than 90% ozone) under field conditions or simulated field conditions. The effects of chronic exposure on agroecosystems is also discussed to a limited extent in Chapter 11.

H. W. Smith[64] has suggested three classes of ecosystem response to air pollution: those in which vegetation and soils serve only as a sink for pollutants, with no visible injury; those in which some species or sensitive individuals within species are injured and are more subject to

other stresses; and those in which high dosages cause acute morbidity or mortality of some species. Although these classes are convenient for discussion, in most situations they are not clearly separated in space. More often, these classes of response occur along a gradient of decreasing pollutant exposure, as in the San Bernardino Mountains.

Extent and Intensity of Injury to Overstory Trees in the San Bernardino National Forest.--Wert (1969) used aerial photography to determine the extent of oxidant injury to ponderosa and Jeffrey pines in diameter classes larger than 30 cm. Injury was categorized as heavy, moderate, light or negligible, and it generally decreased with distance from the source area. Of the 160,950 acres (64,380 hectares) of ponderosa-Jeffrey type within the forest boundaries, 46,230 acres (18,492 ha) had heavy damage, 53,920 acres (21,568 ha) had moderate damage; and 60,800 acres (24,320 ha) had light or negligible damage. An estimated 1,298,000 trees were affected; of them, 82% were moderately affected, 15% were severely affected, and 3% were already dead.

The term "ponderosa-Jeffrey type" is a general term that includes a mosaic of five subtypes described by McBride on the basis of species dominance.[31a] These subtypes are: ponderosa pine forest, ponderosa pine-white fir forest, ponderosa pine-Jeffrey pine forest, Jeffrey pine forest, and Jeffrey pine-white fir forest. The injury by oxidant air pollutants is most intense in the types dominated by ponderosa pine and less intense in the Jeffrey pine types. In the field plots of these various types, the average area covered by shrubs is only 3.8% in the ponderosa types, but 26% in the Jeffrey pine types.[31a]

Later studies[45, 68, 70] have expressed the amount of injury to ponderosa and Jeffrey pines and associated tree species in permanent study

plots as a numerical score. The range of scores is subdivided into seven categories ranging from dead to no visible symptoms.[45]

In 1974, all tree species at 19 permanent study plots were scored individually by binocular inspection. The data can be obtained from conifers early in the fall, but the most important deciduous species, black oak, was evaluated during 3 days, August 28-31, to prevent confusion of oxidant-injury symptoms with natural autumn senescence of leaves. The injury to black oak as of August 31, 1974, at several representative study sites and the June - August accumulated dose at nearby monitoring stations are shown in relation to the topographic projection of the San Bernardino Mountains in Figure 12-13. Lower scores mean greater injury. The darkened portion of the bar representing oak injury is for leaf chlorotic mottle and interveinal necrosis. A score of 8 means no injury. The remaining portion of the score is the sum of scores for leaf complement, leaf size, and twig mortality, not shown separately. These data suggest that oak shows no visible injury where the accumulated June - August dose does not exceed about 2.5×10^5 $\mu g/m^3$-h from about Snow Valley eastward.

The distribution of ponderosa and Jeffrey pines into various injury classes with respect to the distance of the study site along the gradient of oxidant dose (June - September) is illustrated above the topographic projection in Figure 12-14. It is important to realize that the 1974 distribution into injury classes is also a product of earlier years, when the oxidant concentrations were not always as high as in 1974 (see Figure 12-5). The trend toward greater numbers in the very slight (29-35) and no visible injury (36 and greater) categories is quite evident in the eastern plots receiving lower doses -- e.g., Holcomb Valley (HV). The assumption has been made that ponderosa and Jeffrey pine respond similarly to oxidant. Ponderosa pine is

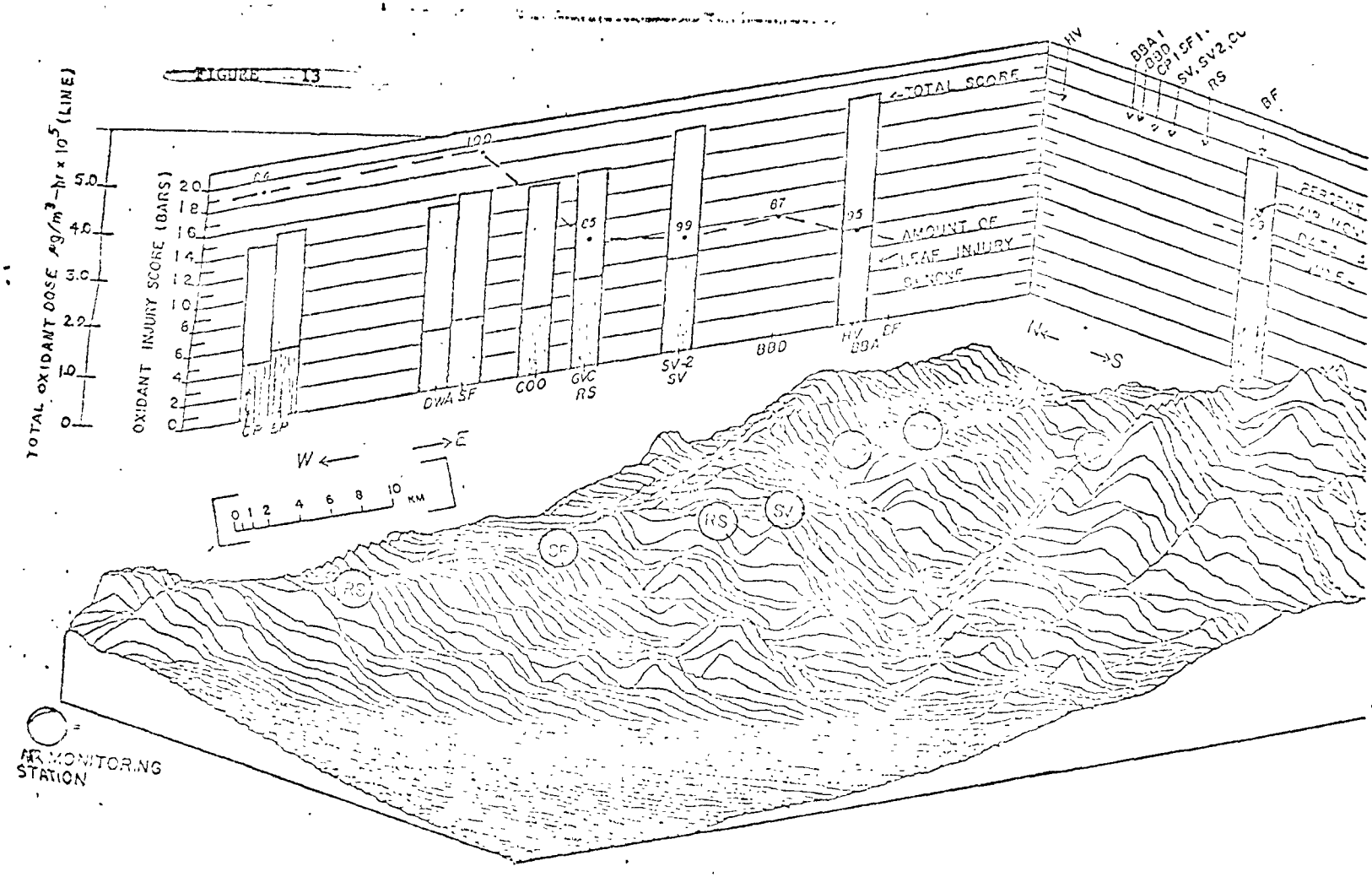

FIGURE 12-13. Topographic projection, San Bernardino Mountains, with comparison of oxidant injury to black oaks at major study sites, August 31, 1974, with accumulated total oxidant dose for June-August measured at nearby monitoring stations. Higher score means less injury. (Reprinted with permission from Kickert et al.[31a])

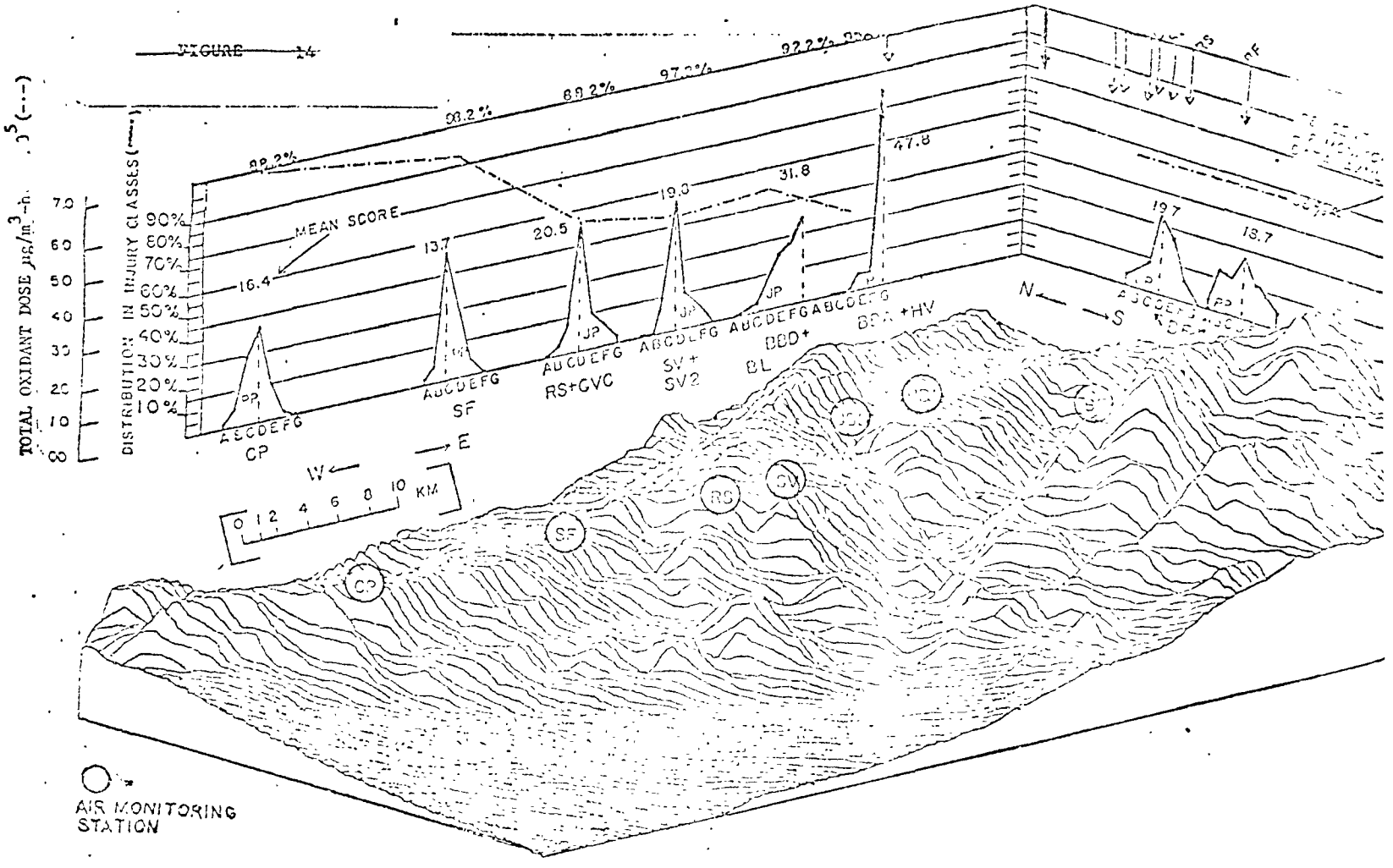

FIGURE 12-14. Topographic projection, San Bernardino Mountains, showing how ponderosa pine (PP) and Jeffrey pines (JP) in major study sites are distributed in six injury classes according to seasonal dose of total oxidant. A, dead, 0; B, very severe, 1-8; C, severe, 9-14; D, moderate, 15-21; E, slight, 22-28; F, very slight, 29-35; G, no visible damage, 36+. (Reprinted with permission from Kickert et al.[31a])

replaced by Jeffrey pine in the natural stands east of Camp O'ongo, and they intermix at Barton Flats (BF). The validity of this assumption can be partially verified by examining the distributions of the two species into injury classes at Barton Flats (Figure 12-14). These data indicate reasonable similarity at a common site; but the influence of other environmental variables that change continuously along the oxidant gradient -- such as soil moisture availability, temperature, and humidity -- must be examined more intensively to understand the degree to which they influence oxidant susceptibility (see Figures 12-8 through 12-12).

Trends in Oxidant Damage to Conifer Species in the 19 Major Study Plots from 1973 to 1974.--The first evaluation of oxidant injury to all tree species in the new study plots was completed in September and October 1973. The second evaluation, in 1974, offered the first opportunity to assess trends of tree injury and mortality. In Table 12-4, the plots are arranged in the order of severe injury (first) to no visible injury (last), according to the 1974 average injury score for all ponderosa or Jeffrey pine in each plot. Of the 12 plots categorized as severe and moderate, all but six showed significantly lower scores (increased injury) with a probability of 0.05. Among the remaining 6 plots classified as slight, very slight, or no visible injury, there were three significant increases, one insignificant increase, and two decreases (one significant and one insignificant), all at a probability of 0.05.

The general increase in injury in the severe and moderate plots is probably related to the 1974 increase in June-September dose (Figure 12-5). Tree mortality among ponderosa and Jeffrey pines was about the same in 1973 and 1974. The largest mortality was at permanent study plots in the moderate injury category. Perhaps the populations in these plots still

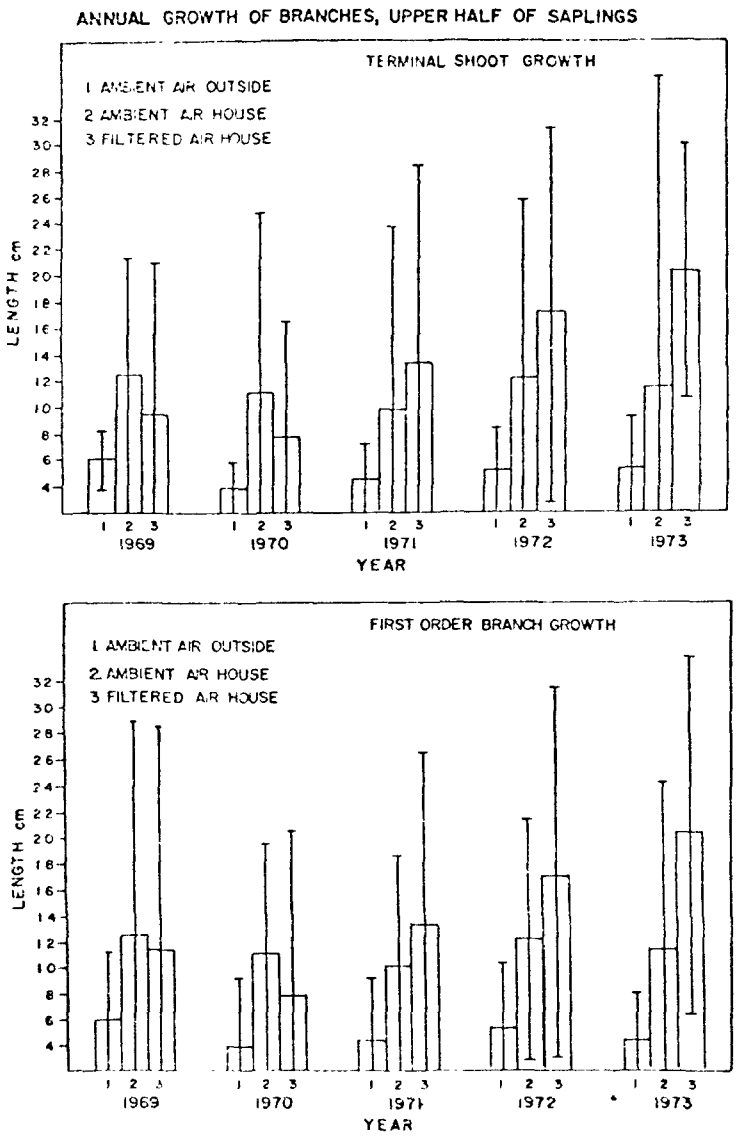

FIGURE 12-15. Annual growth of the terminal shoot (upper) and first-order branches (lower) in upper half of ponderosa pine saplings maintained in filtered or unfiltered (ambient) air greenhouses, and an outside ambient air treatment from 1968 through 1973. (Reprinted with permission from Kickert et al.[31a])

retain greater numbers of the more susceptible genotypes. In earlier years, tree mortality rates for ponderosa or Jeffrey pines in several stands suffering moderate to severe injury were 8% and 10%, respectively, from 1968 to 1972,[68] 8% from 1969 to 1971,[45] and 24% from 1966 to 1969.[10] The final cause of death of weakened trees is usually the pine bark beetle.[65] Mortality has not been observed in tree species other than ponderosa and Jeffrey pine.

Data for the longest observation period of tree decline extend from 1952 to 1972 in two 5-acre (2-ha) control plots in the vicinity of Barton Flats in the San Bernardino National Forest.[80] These plots are in the Jeffrey pine-white fir subtype and are now considered to be in an area of moderate injury (see Barton Flats and Camp Oceola plots in Table 12-4). All Jeffrey pines, with a diameter breast height (dbh) of 12 in. (30.5 cm) and larger were measured and their vigor was described by judging the risk or the probability that they would be susceptible to attack and kill by bark bettles (Dendroctonus sp.). Risk classes 1 and 2 indicate low-risk trees that would definitely be preserved if trees were being marked for a timber sale. Classes 3 and 4 are high-risk trees that would be marked for removal in a timber sale. In Table 12-5, the changes in merchantable volume in board feet (bd ft) in all four classes are recorded for two control plots in 1952, 1963, and 1972. The increases in volume of high-risk trees since 1952 are remarkable; decreases in volume of low-risk trees and total volume in the plots are very large. The total volume decrease is related, first, to one-by-one removal of bark-beetle-killed trees inside the plots indicated for certain by the increase in snags and current stumps and, second, possibly to suppressed radial growth.

One of the objectives of the two 5-acre (2-ha) control plots was to show what would have happened in the absence of sanitation salvage logging. The distributions of trees in all categories in 1952, 1963, and 1972 (Table 12-5) suggest that by 1972 in plots 1 and 2 the sanitation salvage logging

Table 12-4

Changes in Oxidant Injury Scores and Mortality Rates of Ponderosa and Jeffrey Pines at 18 Major Study Plots, 1973-1974

Plot	Species	Tree Density,[a]	Average Injury Score[b]		Mortality Rate, %		Accumulated Mortality, %	Injury Description
		1973	1973	1974	1973	1974	1974	
Schneider Creek (SCR)	JP	28	12.4	11.7	0.0	0.0	0.0	Severe
Camp O'ongo (COO)	PP	90	15.1	12.9*	0.0	0.0	0.0	
Sky Forest (SF)	PP	144	13.3	13.7	0.8	0.8	1.6	
University Conf. Center (UCC)	PP	309	15.5	15.6	0.0	1.5	1.5	Moderate
Breezy Point (BP)	PP	236	16.3	16.0	2.7	2.7	5.5	
Camp Paivika CP)	PP	217	17.1	16.4	0.0	3.1	3.1	
Dogwood A (DWA)	PP	168	19.9	16.5*	1.2	0.0	1.2	
Tunnel Two Ridge (TUN 2)	PP	122	19.5	16.7*	0.0	1.4	1.4	
Camp Angeles (CA)	PP	112	25.6	16.8*	0.0	1.5	1.5	
Barton Flats (BF)	PP	200	21.4	18.7*	3.7	3.7	7.4	
Barton Flats (BF)	JP	124	21.0	19.7	3.6	3.6	7.3	
Snow Valley 2 (SV 2)	JP	129	22.1	19.7*	0.0	1.0	1.0	
Green Valley Creek (GVC)	JP	43	21.8	20.5*	0.0	1.5	1.5	
Camp Oceola (CAO)	JP	192	21.7	22.8*	0.8	7.2	8.0	Slight
Bluff Lake (BL)	JP	186	29.4	31.8*	0.0	0.0	0.0	Very slight
N.E. Green Valley (NEGV)	JP	120	33.1	32.1	0.0	0.0	0.0	
Heart Bar (HB)	JP	130	44.0	39.2*	0.0	0.9	0.9	No visible symtoms
Sand Canyon (SC)	JP	56	41.3	47.3*	0.0	0.0	0.0	
Holcomb Valley (HV)	JP	193	46.4	47.7	0.0	0.0	0.0	

[a] Number of trees per hectare.

[b] Asterisk indicates difference significant at probability of 0.05 (comparisons valid only between years at a single plot).

Table 12-5

Changes of Timber Volume and Percentage of Total Jeffrey Pines in Four Insect Risk Classes at Two Control Plots Excluded from Sanitation Salvage Logging Between 1952 and 1972 at Barton Flats in the San Bernardino National Forest

Risk Classes	Control Plot 1 (JCA Camp, Highway 38)					
	1952		1963		1972	
	Timber Volume, bd ft	Percentage of Trees	Timber Volume, bd ft	Percentage of Trees	Timber Volume, bd ft	Percentage of Trees
Total, all classes	73,040	100	63,530	100	52,730	100
Risks 1 and 2	58,520	87	38,700	73	23,780	55
Risk 3	6,740	7	14,630	13	14,140	16
Risk 4	7,780	5	10,200	7	14,810	20
Snags and current stumps[a]	1	1	11	7	13	8
	Control Plot 2 (Camp Oceola Road)					
	1952		1963		1972	
Total, all classes	120,130	100	112,660	100	112,930	100
Risks 1 and 2	110,830	93	98,080	82	45,670	32
Risk 3	5,990	3	10,170	6	37,420	30
Risk 4	3,310	2	4,410	6	29,840	28
Snags and current stumps[a]	3	2	13	6	18	10

[a] Accumulation during 10-year period. Data obtained from the Supervisor's Office, San Bernardino National Forest.

or timber sales would have removed 45% and 68% of overstory trees, respectively. The more selective tree-by-tree salvage would have removed only 8% and 10% of the overstory trees in plots 1 and 2, respectively. These results must be treated as suggestions, not firm conclusions, because of the lack of a more adequate statistical design, which should have included more plots in additional stand types and sampling of several kinds of sites at various distances from the pollution source. However, because of the suggested impact, the sanitation salvage logging practice needs careful scrutiny. Other information is available from the ponderosa pine subtype that suggests that biomass production of overstory species is diminished in proportion to the oxidant dose received. Parmeter et al.[54] observed decreases in height growth of ponderosa pine that showed injury symptoms. Injured trees did not respond with greater growth during years with more favorable soil moisture content; uninjured trees (often side by side with injured trees) did have increased height growth in these years. McBride (in Kickert et al.[31a]) studied two populations, each including 19 ponderosa pines dominant in their stands. One population ranged in age from 52 to 71 years in 1972, and the other, from 20 to 39 years. The influence of tree age on ring width growth was minimized by comparing rings of equivalent age in each population. The measured rings in the older group were produced from 1910 to 1940, before the advent of "Los Angeles smog," and in the younger group, from 1941 to 1971, when smog was present. After the influence of precipitation on growth was evaluated, there was a difference of 0.20 cm in average annual growth attributable to oxidant air pollutant injury. In this sample, a 30-year-old tree subjected to air pollution would be 24 ft (7.2 m) tall and 7.5 in. (19 cm) in dbh, with one 16-ft (48-m) log having a volume of 30 bd ft. In comparison, a 30-year-old tree growing

before the injury by oxidant air pollutants (1910-1940) would be 30 ft (9 m) tall and 12 in. (30.5 cm) in dbh and could yield a 4.8-m log with a volume of 80 bd ft. The impact of oxidant stress on forest growth is apparent without further eleboration.

The growth suppression of ponderosa pine saplings has been demonstrated by enclosing a group of 10 naturally seeded trees in the plastic-covered greenhouses used by Thompson;[72] enclosed trees were provided with activated-charcoal-filtered air (FAH) from April through October 1968 - 1973 by Miller and McBride (in Kickert et al).[31a] The controls consisted of two similar groups of trees, one in a greenhouse receiving unfiltered or ambient air (AAH) and another group not enclosed (AAO). Precipitation was rare during the summer enclosure period, and all three groups received the same amount of winter precipitation. At the end of the 1973 growing season, most of the saplings were harvested from each treatment for growth analysis. One indicator of the response to these treatments was the length of annual growth of terminal shoots and first-order branches in the upper half of the saplings (Figure 12-15). After an initial lag in 1969 and 1970, the growth of shoots and branches on the FAH trees increased dramatically. The retention of annual needle whorls was remarkably different by 1973; needles in internodes older than 1972 were completely absent from the AAH and AAO trees. The decrease in needle biomass on the AAH and AAO trees for the 1973 whorl was 32 and 37%, compared with that on FAH trees (Figure 12-16).

Needle length and litter production furnish another important measure of biomass decrease. In field studies, it was found that the average amount of litter production increased with decreasing ozone injury of selected ponderosa and Jeffrey pines ($r = 0.55$) and that the average mass of needles per fascicle increased with decreasing ozone injury ($r = 0.96$).[70]

Early studies[65] suggested that injury to ponderosa pine was similar in all size classes, but Cobb and Stark[10] later reported higher mortality

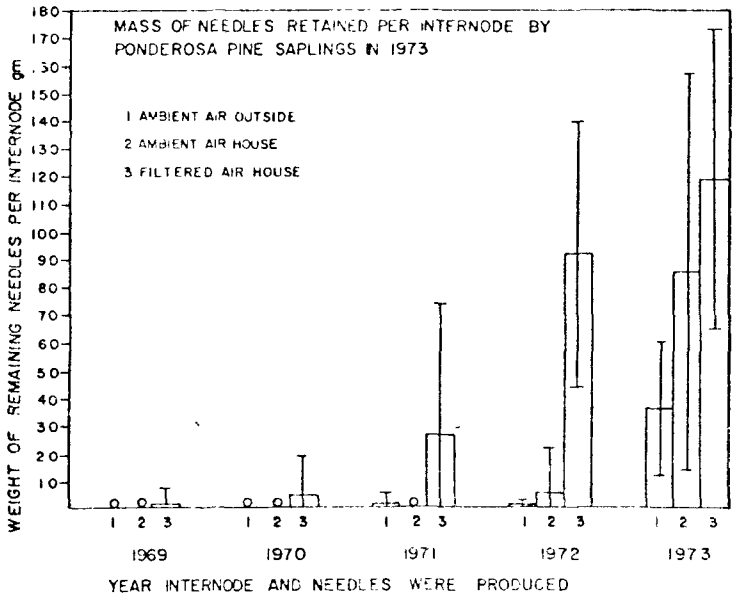

FIGURE 12-16. Average weight of all needle fascicles retained in 1973 per internode in filtered or unfiltered (ambient) air greenhouses, and an outside ambient air treatment after treatment since 1968. (Reprinted with permission from Kickert *et al.*[31a])

rates in understory ponderosa pines (9-12 in., or 22.9 - 30.5 cm, in diameter) than in larger size classes. The probable effect of tree mortality on stand composition can be anticipated to some extent from an example of the present species and size-class composition, as shown in Table 12-6.[45] In this severely damaged stand in the ponderosa pine-white fir subtype, nearly 50% of the overstory and about 22% of the understory is ponderosa pine. With all size classes considered, 16.1% had slight, 33.3% moderate, 31.2% severe, and 19.4% very severe injury. White fir and incense cedar are well established in the understory, but their poorer survivorship to 12.00-23.99 in. (30.5 - 60.9 cm) in dbh compared with ponderosa pine suggests that other mortality factors have acted heavily on them in the past. Incense cedar and sugar pine are the most tolerant to oxidant, but sugar pine is present in very low numbers. The accelerated mortality of ponderosa pines has particular significance in this ecosystem, because it is the dominant member of the climax community.

The direct effects of ozone on plant species constituting the shrub layer in the conifer forest are not yet sufficiently understood to permit any conclusion to be drawn. In many sites where the conifer overstory is well developed, the shrub species are excluded completely. In more open pine stands, some shrubs are very common, such as skunk bush (Amorpha californica) and white thorn (Ceanothus cordulatus). Of these two species, only skunk bush shows highly visible chlorotic mottle of leaflets and premature defoliation where ozone dosages are high. A shrub species common to the lower chaparral zone, squaw bush (Rhus trilobata), is even more susceptible to ozone injury.[57] In the San Bernardino Mountains, 166 herb-layer species have been identified as common to the conifer forest.[70]

Table 12-6[a]

Tree Species and Size Composition on a Study Site Affected by Oxidant Air Pollution[a]

Tree Size Class	Ponderosa Pine	Incense Cedar	White Fir	Sugar Pine
	Number/Acre			
Understory				
Seedlings (up to 3.00 ft tall)	1057	2381	1043	302
Saplings (more than 3.01 ft tall, less than 3.99 inches dbh)	33	33	57	10
Poles (4.00 to 11.99 inches (dbh)	21	12	38	3
Percent:	22.2	48.6	22.8	6.3
Overstory				
Standard (12.00 to 23.99 inches dbh)	18	9	8	3
Veteran (24 inches dbh and larger)	12	5	4	2
Percent:	49.6	22.7	19.7	8.0

Trees from 575-acre study area, San Bernadino National Forest, Calif.

[a] Reprinted with permission from Miller.[45]

The phenology of these species is being studied so that growth and flowering can be correlated with soil moisture availability and seasonal changes of ozone dose. Initial field observations suggest that at least 10 species are obviously injured by total oxidant in areas receiving high dosages. These areas have been subject to exposure for at least 20 years, so some species or subspecies may have been completely eliminated. Dunn[18] reported that smog may have acted as a selective agent to remove oxidant-sensitive subspecies of Lupinus bicolor from the Los Angeles area. Furthermore, L. latifolius appeared to have floral ontogeny inhibited without damage to leaves, and L. densiflorus was severely damaged by ambient total oxidant at Claremont, California.

Price[56] selected six important grass species found in either the Gambel oak or quaking aspen communities in the Wasatch Mountains of Utah. The selected species were fumigated under greenhouse conditions with ozone varying from 0.15 to 0.30 ppm during each experiment. These treatments caused a 53% reduction in top-growth biomass of the six species. The initiation of reproductive structures of all species was inhibited, and the reproductive potential of Bromus tectorum and B. carinatus declined to zero. These results suggest that the species composition of affected natural communities could be altered. In other studies, 17 representative species from the quaking aspen community were fumigated with ozone 3 h each day, 5 days/week, with ozone at 0.30, 0.15, and 0.05-0.07 ppm in the first two cases and with ambient total oxidant in the last case. Experiments extended through three seasons. At concentrations of 0.15 ppm or below, growth reduction and stimulation were both observed in different species; growth was reduced in all but 2 species at 0.30 ppm. Seed production was reduced in 2 species at 0.15 ppm and in 3 species at 0.30 ppm, of the total of 6 species that produced fruits or seeds.[25]

In a third series of experiments, Treshow and Stewart[77] fumigated native plants growing naturally under field conditions. The threshold ozone concentrations required to injure important species in the grassland-oak and aspen-conifer communities during 2-h exposures were determined (Table 12-7). Three important species -- Bromus tectorum, Quercus gambelli, and Populus tremuloides -- were injured by a single 2-h exposure to ozone at 0.15, 0.25, and 0.15 ppm, respectively; over half the remaining species showed visible injury after exposure to 0.30 ppm or less. The implications for possible imbalances in community stability are readily apparent if these plant communities receive dosages similar to those typical in the San Bernardino Mountains.

Yonkers et al.[84] have tested the ozone susceptibility of 15 species of annuals common to the Mojave Desert just north and east of the Los Angeles basin (and San Bernardino Mountains). Compared with the susceptible pinto bean plants included in the experiment with ozone at 0.35 ppm, Plantago sp., Cercidium sp., and Prosopsis sp. were also sensitive. Further interpretation of these results is complicated by the influences of plant age and preexposure conditions.

Dose-Injury Observations with Ambient Oxidant and Native Ponderosa Pine Saplings.--During 1969, 1970, and 1971, 10 sapling-size ponderosa pines that received the ambient air outside (AAO) treatment for comparison with trees enclosed in filtered- or ambient-air greenhouses were observed to determine the rate of current-year needle growth and appearance of oxidant injury symptoms on both current and 1-year-old needles.[31a] Forty new needles on each tree were measured monthly (Figure 12-17, left); growth leveled off in early September. Current-year and 1-year-old needles were

Table 12-7[a]

Injury Thresholds for 2-hours Exposures to Ozone

Species	Injury threshold (pphm ozone)
Grassland-Oak Community Species	
Trees & Shrubs	
Acer grandidentatum Nutt.	over 40
Acer negundo L.	over 25
Artemisia tridentata Nutt.	40
Malosma laurus G.Don	over 40
Potentilla fruticosa L.	30
Quercus gambelii Nutt.	25
Toxicodendron radicans (L.) Kuntze	over 30
Perennial Forbs	
Achillea millefolium L.	over 30
Ambrosia psilostachya DC.	over 40
Calochortus nuttallii Torr.	over 40
Cirsium arvense (L.) Scop.	40
Conium maculatum L.	over 25
Hedysarum boreale Nutt.	15
Helianthus annus L.	over 30
Medicago sativa L.	25
Rumex crispus L.	25
Urtica gracilis Ait.	30
Vicia americana Muhl.	over 40
Grasses	
Bromus brizaeformis Fisch. & Mey.	30
Bromus tectorum L.	15
Poa pratensis L.	25
Aspen and Conifer Community Species	
Trees & Shrubs	
Abies concolor (Gord. & Glend.) Lindl.	25
Amelanchier alnifolia Nutt.	20
Pachystima myrsinites (Pursh) Raf.	over 30
Populus tremuloides Michx.	15
Ribes hudsonianum Richards.	30
Rosa woodsii Lindl.	over 30
Sambucus melanocarpa A.Gray	over 25
Symphoricarpos vaccinioides Rydb.	30
Perennial Forbs	
Actaea arguta Nutt.	25
Agastache urticifolia (Benth.) Kuntze	20
Perennial Forbs	
Allium acuminatum Hook.	25
Angelica pinnata S.Wats.	under 25
Aster engelmanni (Eat.) A.Gray	15
Carex siccata Dewey	30
Cichorium intybus L.	25
Cirsium arvense (L.) Scop.	under 40
Epilobium angustifolium L.	30
Epilobium watsoni Barbey	30
Eriogonum heracleoides Nutt.	30
Fragaria ovalis (Lehm.) Rydb.	30
Gentiana amarella L.	over 15
Geranium fremontii Torr.	under 25
Geranium richardsonii Fisch. & Traut.	15
Juncus sp.	over 25
Lathyrus lanzwertii Kell.	over 25
Lathyrus pauciflorus Fern.	25
Mertensia arizonica Greene	30
Mimulus guttatus DC.	over 25
Mimulus moschatus Dougl.	under 40
Mitella stenopetala Piper	over 30
Osmorhiza occidentalis Torr.	25
Phacelia heterophylla Pursh	under 25
Polemonium foliosissimum A.Gray	30
Rudbeckia occidentalis Nutt.	30
Saxifraga arguta D.Don	under 30
Senecio serra Hook.	15
Taraxacum officinale Wiggers	over 25
Thalictrum fendleri Engelm.	over 25
Veronica anagallis-aquatica L.	25
Vicia americana Muhl.	over 25
Viola adunca Sm.	over 30
Annual Forbs	
Chenopodium fremontii Wats.	under 25
Collomia linearis Nutt.	under 25
Descurainia californica (Gray) O.E.Schulz	25
Galium bifolium Wats.	over 30
Gayophytum racemosum T. & G.	30
Polygonum douglasii Greene	over 25
Grasses	
Agropyron caninum (L.) Beauv.	over 25
Bromus carinatus Hook. & Arn.	under 25

[a] Reprinted with permission from Treshow and Stewart.[77]

inspected monthly for amount of chlorotic mottle, necrosis, and abscission. Each symptom was given a descriptive rating: 0, none; 1, very slight; 2, slight; 3, moderate; and 4, severe. In Figure 12-17, an average of the 3 years shows that, by Julian day 250 (September 7), the current-year needles had a visible-injury score of 2.2, indicating slight chlorotic mottle (signifying severe injury to the trees); there was usually no necrosis and almost always no abscission of current-year needles. Symptoms developed more rapidly on 1-year-old needles; the combined score of the current-year and 1-year-old needles was about 9.

The accumulated ozone dose since June 1, which is associated with the current-year and 1-year-old needle injury expressed on the left of Figure 12-17 can be roughly estimated by transferring the injury score to the right. For example, the current-year needle score of 2.2 on September 7 is associated with a total oxidant dose of 2.75×10^5 $\mu g/m^3$-h. These results assume that all the air monitoring data were available, but on the average about 90% were available. The dose-injury curve is apparently not linear as assumed, and interpretation is further confounded by the random occurrence of injurious doses in the field. Additional observations of ponderosa and Jeffrey pine are required to define more completely the ozone or total oxidant dose response. The development of a model relating injury to dose and important controlling environmental variables is a high-priority need for understanding chronic-exposure effects.

Effects on Reproduction

The effect of ozone injury on herbaceous plant reproduction has been mentioned earlier in this chapter and in Chapter 11. Seed production by annuals is influenced mainly by the environmental conditions of the current

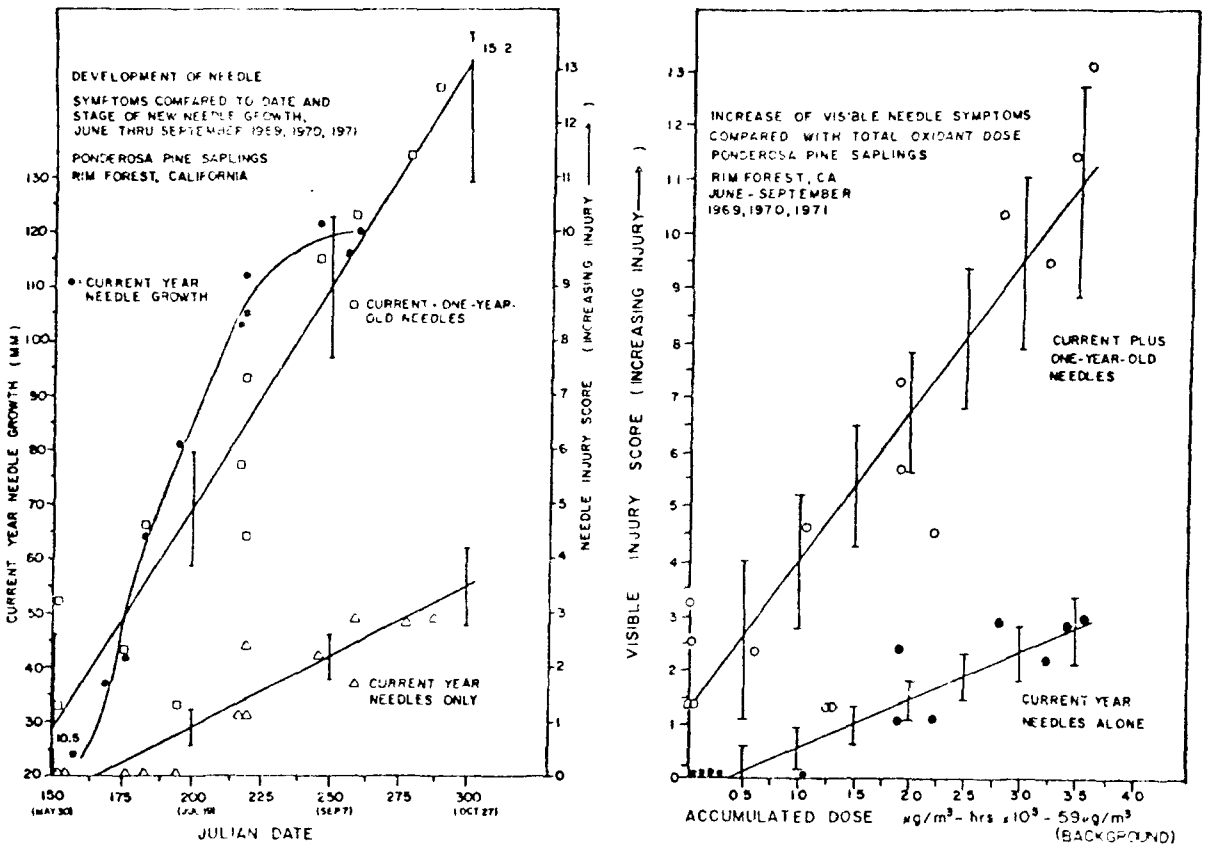

FIGURE 12-17. Development of oxidant injury symptoms on current and current-plus-1-year-old needles of ponderosa pine saplings, in relation to stage of current-year needle growth and time during the summer season (left) and in relation to total dose of oxidant (right). (Reprinted with permission from Kickert et al.[31a])

year, but perennial woody plants -- particularly conifers -- are erratic seed-producers. Intrinsic factors affecting cone production include age and vigor; seasonal temperature and soil moisture are important environmental factors.[22]

The effects of sulfur dioxide on cone production have been described by Scheffer and Hedgecock[60] and Pelz.[55] Generally, the decrease in tree vigor caused by the pollutant may eliminate or lower the frequency of cone production and diminish the size, weight, and germination of seed. The effects of chronic ozone injury on conifer seed production may be similar, in that tree vigor is drastically reduced. The effects of chronic ozone injury on ponderosa and Jeffrey pine seed production are under investigation by Luck (in Kickert at al.[31a])

Seeds from individual species may constitute the bulk of the diet of small vertebrates, such as the deer mouse (Peromyscus sp.) and the western gray squirrel (Sciurus griseus anthonyi). During periods of low seed production due to diminished tree vigor, squirrels converge on the few remaining vigorous ponderosa pines and consume about half the seed crop before it matures and reaches the ground. In the areas severely affected by ozone, squirrels return to the same trees year after year. After the seed reaches the ground, other small vertebrates, such as mice, seek it out. The habit of preferential seed use by small vertebrates may be a stress acting additively with ozone injury to decrease seriously the regeneration potential of ponderosa pine.

Indirect Effects

Most indirect effects of oxidant air pollutants on primary production and reproduction would be mediated through changes in the physical environment resulting from disruption of stand structure. For example, Hursh[28]

described three microclimates in the area surrounding the ore smelter at Copper Basin, Tennessee. These zones were distinguished by changes in air and soil temperatures, winds, evaporation, air moisture, and rainfall. Because sulfur dioxide was the pollutant in this case, there were also changes in soil pH and sulfur content, which would not accompany oxidant air pollution damage. Soil erosion increased dramatically.

Changes in such physical factors as light, temperature (particularly maximums and minimums), relative humidity, and wind speed in forest communities subject to structural alteration by mortality of susceptible species could change the suitability of some sites for growth, reproduction, and reestablishment of survivor species. We can only speculate on some of the possible secondary effects until more data are gathered.[45]

Wildfire is a very important factor in western forest ecosystems. In the San Bernadino Mountains, the fire frequencies were determined by J. R. McBride and R. Laven (in manuscript) in two stand types before and after 1893, when the area was first set aside as a forest preserve and fire protection began. Before 1893, the average interval between fires in ponderosa stands was 12 years; after 1893, it was 24 years. The comparable numbers for Jeffrey pine stands were 16 and 38 years. The buildup of heavy fuels due to ozone-caused mortality and fire protection results in hotter fires, and the thinning of the tree canopy results in increased rates of fire spread.[14] Hotter fires decrease tree survival. Moisture interception by condensation in living tree crowns would decrease as the stands became thinner, thus causing some sites to be drier.[32]

EFFECTS ON CONSUMER POPULATIONS

Vertebrate Populations

The effects of oxidant air pollutants on vertebrates can be segregated into direct and indirect categories. Direct effects are clinical

and pathologic alterations of tissues that result from exposure to ambient air. Indirect effects result from alterations in numbers or distribution of the plant and animal populations exposed to ambient air. For example, if air pollution eliminates or thins numbers of a susceptible plant species, the food chain of the consumers that feed on it may break down. The result could be a simpler and less stable ecosystem, with fewer plants and animals in species and numbers.

The clinical and pathologic effects of oxidant air pollutants on domesticated vertebrates have received little study in the laboratory. We have found no major references to studies of these effects on free-ranging native species. It is therefore necessary to extrapolate from laboratory results to probable effects on wildlife in the field and forest.

Chapter 8 indicates that oxidant air pollutants may adversely affect the olfactory and sight senses, degree of activity, general health and vigor, reproductive rate, heart and kidney function, protein synthesis, respiratory function, and disease resistance in domestic vertebrates in laboratory conditions. Many of these adverse physical responses develop at or near the concentrations of ozone currently experienced daily in ambient air in parts of the San Bernardino Mountains.

Although it is risky to extrapolate from domestic species in a laboratory to free-ranging species in the forest, it appears likely that some wild vertebrates in the San Bernardino Mountains could be affected adversely by their prolonged exposure to current concentrations of ozone. Nitrogen dioxide, PAN, ozone, and environmental factors may act additively or synergistically to increase the overall stress on the individual. Potentially, the direct effects of oxidant air pollution on wild vertebrates within a forest system could be great. A reduction in visual or olfactory acuity or other

loss of health or condition could be a serious handicap for a predator whose survival depends on overcoming prey, and equally serious for the prey that survives by staying one jump ahead of a predator.

In the San Bernardino Mountains, our goals have been to describe the terrestrial vertebrate community within this mixed-conifer forest, particularly in relation to ponderosa and Jeffrey pine stands, and to determine the effects of oxidant air pollutants on this community. The possible interactions of vertebrates in this system are shown in Figures 12-9 through 12-12.

The most abundant species of small mammals were selected to exemplify vertebrate interactions, because of the importance of this group as seed-eaters. The results from trapping of abundant species show that the deer mouse (Peromyscus spp.) is the most numerous. Chipmunks (Eutamias spp.), the golden-mantled ground squirrel (Callospermophilus lateralis), the dusky-footed woodrat (Neotoma fuscipes), the meadow mouse (Microtus californicus), and the harvest mouse (Reithrodontomys megalotus) are the other common small mammals.[33] Numbers have fluctuated widely from year to year and from plot to plot, as is characteristic of small-mammal populations. Deer mouse numbers fluctuate the most. Preliminary analysis indicates that the same species of small mammals are present in this forest as were reported from similar trappings 70 years ago.[33] Thus, it appears that oxidant air pollution has not yet resulted in a reduction in the diversity of this component of the vertebrate fauna. Population numbers of the common small-mammal species do, however, appear to be low, in comparison with other similar forest regions.[31a] This is particularly true for the study plots in areas of heavy oxidant air pollution. We have not yet determined whether these low population densities of small mammals are directly or indirectly related in any measure to the oxidant air pollution. These differences

may result from variations in other aspects of habitat quality. For example, the study plots in the areas of heavy oxidant air pollution are dominated by ponderosa pine and have sparse shrub cover, whereas the study plots in areas of light oxidant air pollution are dominated by Jeffrey pine and have much more shrub cover.[31a]

When changes occur in one part of an ecosystem, the intimate nature of the interrelationships results in changes in many other parts. Any factor that causes change in one component of a system potentially affects all subsystems of that ecosystem. The most important indirect effects of oxidant air pollutants on vertebrates are those resulting in changes in the habitat. Foremost among these effects are those on the vegetation and the successional patterns of the plant community. Because of the high degree of interrelationship and interaction between the vegetation, the fauna, and the inorganic matrix of an ecosystem, effects of air pollution on the vegetation potentially can result in changes throughout the ecosystem. Damage to vegetation is probably the most important effect of chronic, low-concentration air pollution on wildlife. Ponderosa pine, Jeffrey pine, and black oak are all susceptible to damage, and these are the most important trees within the forest as providers of food and habitat for wildlife. A similar selectivity by species doubtless occurs within the shrub and herb layers of the vegetation. The long-term effects will be reduced production of fruits and seeds and elimination of the sensitive plant species and, therefore, reduction in the diversity of the vegetation. In turn, this will lead to a reduction in abundance and diversity of the vertebrate fauna.

Likewise, Woodwell's prediction[83] of enhancement of the activity of insect pests and some disease agents (which has been demonstrated on this forest) could lead to an increase in vertebrate species that feed on invertebrates or utilize dead plants for cover. Birds would be the

most likely to increase and, to a lesser extent, such small mammals as deer mice, which are partially insectivorous.

Fruit and seeds make up the largest part of the diet of most of the common small mamals on our study sites, particularly the deer mouse, harvest mouse, chipmunk, ground squirrel, and western grey squirrel (Sciurus griseus). The grey squirrel is an excellent example of the interactions within this forest and of the potential effects of oxidant air pollution. It is abundant throughout the mixed-conifer type, depending specifically on the pines and oaks for the majority of its food, cover, and nest sites. This squirrel eats or stores a major portion of the pine and oak seed crops each year. On some yellow pine trees on our study plots, grey squirrels cut more than 2,000 cones per tree.

Thus, tree squirrels are a major source of loss of seeds of ponderosa, Jeffrey, and sugar pine and of black oak acorns. Vertebrates, then, can have a major effect on the reproduction of these species, particularly because the grey squirrel is only one of numerous species in this forest that feed on conifer seeds and acorns.

An alteration of the balance between pine and oak, a change in squirrel populations, or a reduction in any of the mast crops brought about by oxidant air pollution obviously could have a major impact on this forest, particularly on tree reproduction and successional patterns. Reductions in conifer seed crops as a result of air pollution has been suggested in other forests, where cone production was lowered and size, weight, and germination ability of the seeds were reduced.[55, 60]

Another way that oxidant air pollution could affect this subsystem is through an alteration in forest moisture. Elimination of vegetation cover allows the exposed soil to dry more rapidly, which would affect soil-burrowing and soil-inhabiting vertebrates. The lower moisture content may reduce or

inhibit fruiting-body formation of fleshy fungi. These fleshy fungi are an important food source for tree squirrels, making up a third or more of their diet in some seasons. A reduction in this food source doubtless would result in an even greater utilization of conifer seeds and acorns, reducing further the reproductive capabilities of these trees and eventually limiting future food supplies for the squirrel population.

In summary, subtle and simple initial changes may radiate and magnify throughout all trophic levels of the ecosystem. Restoration of the system may be impossible.

Macroarthropods

Laboratory studies have indicated that ozone at 196 $\mu g/m^3$ (0.10 ppm) was lethal to adult houseflies (Musca domestica L.) and caused them to lay fewer eggs.[2] Two cockroach species (Paraplaneta americana L. and Nauphoeta cinerea, Oliver) and the red fire ant (Solenopsis invicta Buren) were exposed to ozone at 588 $\mu g/m^3$ (0.30 ppm) for up to 10 days. There was no unusual mortality or evidence of direct injury to individual insects. The fire ant workers were stimulated to migrate inside their nest initially, but further observations indicated no disruption of social behavior.[36] These reports do not suggest that free-ranging insects would be directly affected by ambient concentrations of ozone in natural ecosystems or agroecosystems.

The indirect effects of ozone through modification of the availability of food for insects, particularly in a conifer-forest ecosystem, has received some investigation.[65, 66] The weakening of ponderosa pines by chronic exposure to ozone makes them more vulnerable to successful infestation by pine bark beetles (Dendroctonus brevicomis and D. ponderosae). Figure 12-18 shows a positive relationship between degree of tree injury and frequency of bark beetle infestation and the relative frequency of attack

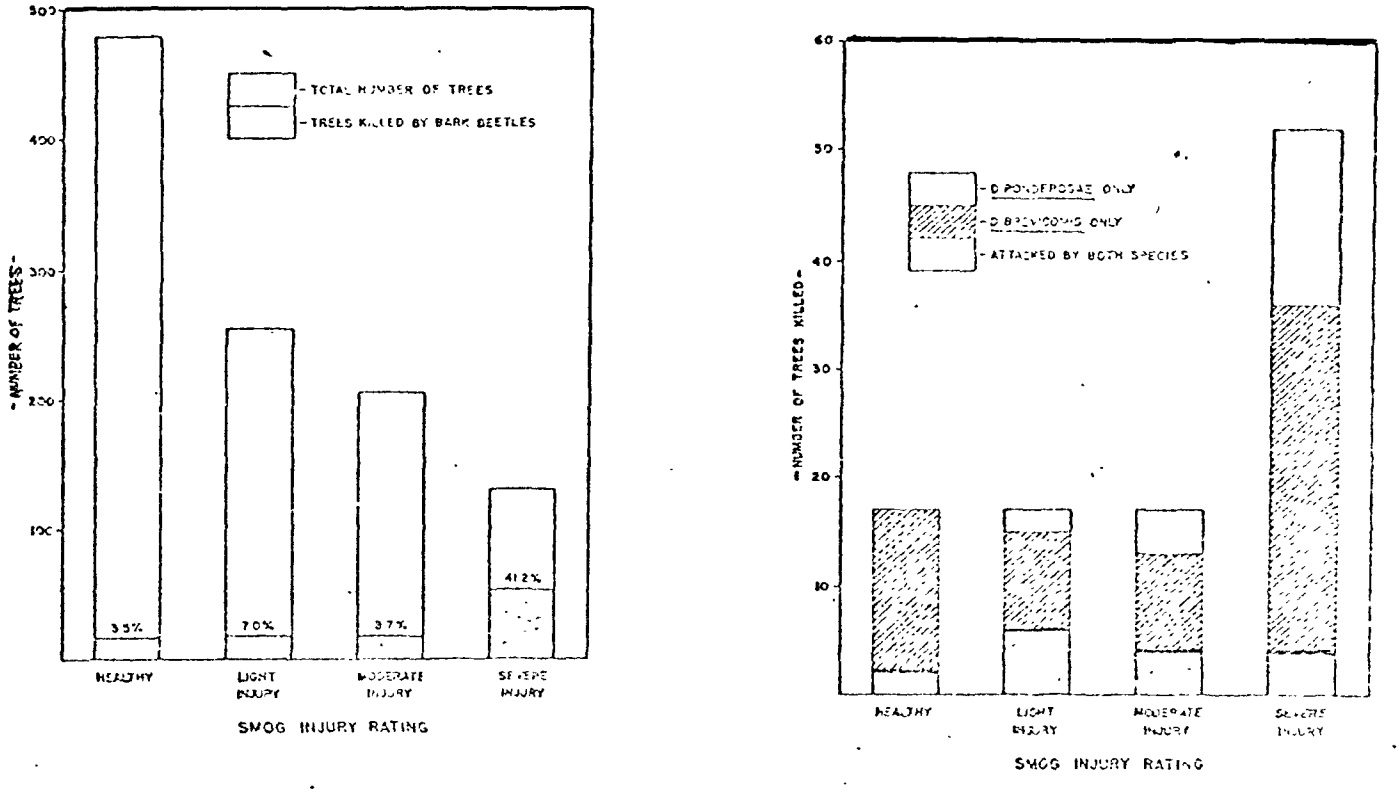

FIGURE 12-18. Relationship between degree of oxidant injury to ponderosa pines and bark-beetle attack (left) and numbers of trees killed by western pine beetle, mountain pine beetle, of the two species together (right). (Reprinted with permission from Stark and Cobb.[66])

by the two species of bark beetles.[66] The relationship (see Figures 12-9 through 12-12) between tree health and brood productivity and the population dynamics of bark beetles and their insect associates in infested trees are being investigated.[31a, 70] Pine bark beetles have been a constant threat to ponderosa pines in the San Bernardino Mountains for many years, since before the inception of oxidant air pollution injury.[67] Bark beetles are a key element responsible for accelerating the modification of stand structure.

Plant Parasites and Symbionts

Parasitic Phanerogams. Both true mistletoe and dwarf mistletoe are common parasites of forest tree species. The true mistletoes (Phoradendron sp.) occur commonly on California black oak and white fir and less often on incense cedar in the San Bernardino Mountains. No direct effects of oxidants have been noted on the mistletoe plant itself under field conditions. The true mistletoe obtains mainly water from its host and would be indirectly affected by debilitation of the host tree. The dwarf mistletoes (Arceuthobium sp.) are common on ponderosa, Jeffrey, and sugar pines in the San Bernardino National Forest. They depend on their host for both water and carbohydrates. Heavily infected or "broomed" branches on ponderosa or Jeffrey pines severely injured by ozone often have more annual needle whorls retained than do uninfected branches on the remainder of the tree. The needles are also greener. It can be hypothesized that the infected branch is a carbohydrate sink where a pooling of carbohydrates occurs; higher carbohydrate concentrations may be instrumental in either preventing or helping to repair ozone injury to needles on the broomed branches. In the long term, stresses from mistletoe and ozone are probably additive and hasten tree death.

Parasitic Fungi. Fungi that infect above-ground plant parts causing leaf or stem diseases may be directly affected by ozone, as discussed in Chapter 11.

Ozone injury to needles of eastern white pine increased infection by Lophodermium pinastri and Pullularia pullulans.[12] These results suggest that leaf tissue of many species may be made susceptible to fungi that are normally saprophytes but may be low-grade parasites when circumstances permit.

In the San Bernardino National Forest, the needle- and twig-infecting fungus Elytroderma deformans is a common fungus disease of ponderosa and Jeffrey pines. The additive stresses of ozone and fungus on the host may hasten tree decline in severe cases. In some situations, it appears that infected or broomed twigs may die sooner than uninfected twigs at the same position in the tree. Observations are continuing.[31a]

Root-infecting fungi, such as Armillaria mellea and Fomes annosus, are generally more virulent pathogens when they encounter trees already weakened by other stresses. This observation has been made mostly in Europe, where sulfur dioxide was the principal pollutant; the spread and virulence of F. annosus across the gradient of decreasing oxidant dosage are being studied in the San Bernardino Mountains.[31a]

Significant increases have been reported in length of root tissue colonized by annosus when artificially inoculated ponderosa and Jeffrey pine seedlings were fumigated with ozone at 431 $\mu g/m^3$ (0.22 ppm) and 888 $\mu g/m^3$ (0.45 ppm) 12 h/day for 58 and 87 days.[31a]

Beneficial mycorrhizal fungi infect the small feeder roots of trees and other plants. The resulting relationship is symbiotic and involves an intimate exchange of minerals and essential metabolites. The host tree benefits through increased efficiency of nutrient uptake from the soil. Any interruption or imbalance of the exchange of materials between the host root tissue and the fungus mantle surrounding it can have deleterious effects on the fungus and the host. Such stresses as air pollution injury to the host undoubtedly disrupt this balance.[24] The feeder rootlet system of ponderosa pines in the San Bernardino Mountains and those of eastern white pine have

shown remarkable deterioration involving diminished numbers of mycorrhizal rootlets, which are replaced by saprophytic fungi that decay smaller rootlets.[54]

EFFECTS ON DECOMPOSERS

Although some of the solar energy fixed by producing plants is released by the respiration of these plants and of animals, much of it is stored in dead organic matter until released by decomposer organisms at rates that vary greatly with place, season, and kind of organic matter. Generally, one-third or more of the energy and carbon annually fixed in forests is contributed to the forest floor as litter, mostly leaves.[53] Because litter is generally related to the quantity of photosynthetic tissue in the ecosystem, it is a useful index of ecosystem productivity.

One of the predicted effects of pollutants on ecosystems suggested by Woodwell[83] is a reduction in the standing crop of organic matter, which would lead to a reduction in nutrient elements held within the living system. The evidence discussed earlier definitely shows that primary production is much lower in an ozone-stressed conifer-forest ecosystem. This result would be anticipated in all similarly stressed natural ecosystems or agroecosystems.

The reservoir of energy and mineral nutrients represented by litter is a very important resource in natural ecosystems with closed nutrient cycles. The growth of new green plant tissue depends on the slow release of nutrients by decomposer organisms. In agroecosystems geared for high production, litter is often removed or burned, and fertilizer is added to the soil; the nutrient cycle is open and subsidized.

In a conifer forest, litter production and decomposition release about 80% of the total minerals in the biomass of the stand; the remainder is retained in the living parts of the tree.[41] Standing dead material is not considered litter.

In terrestrial ecosystems, most decomposers occupy the mantle of litter or the surface layers of the soil, where they supply the necessary recycling mechanisms to convert dead plant or animal material into humus and eventually into minerals, gases, and water. Small animals, arthropods, fungi, and bacteria exist as a complex in intricate food chains in which they feed not only on dead material, but also on one another, ultimately releasing the mineral nutrients needed by the producer populations. Without the decomposers, some essential elements -- such as calcium, phosphorus, and magnesium -- would concentrate in the litter until the supply in the soil was depleted; growth of green plants would then be seriously limited.

Direct Effects

The decomposition of tree leaves is not entirely confined to the litter layer on the forest floor. Leaves and needles are invaded by bacteria and fungi even as they grow; these microorganisms may be either pathogens or saprophytes.[29]

The first possible interaction between ozone and decomposer organisms might be with the bacteria and fungi that occupy the surface of the living green leaf. The direct effects of ozone on microorganisms are discussed in Chapter 11, but very little conclusive information is available that can be applied to natural conditions.

It is not known whether ozone, PAN, or other oxidants could have any direct influence on decomposer organisms in the litter layer; however, there does appear to be a rapid flux of ozone to soil surfaces. The ozone flux to some kinds of surfaces constituting ecosystems--e.g., vegetation, soil, and water--has been determined by Aldaz;[1] he expressed the flux as molecules per square centimeter per second times $10.^{11}$ The relative fluxes into different surfaces, assuming an ozone concentration of 40 $\mu g/m^3$, were:

fresh water, 0.5; snow, 0.9; grass, 1.1; sand or dry grass, 5; and juniper bush, 10. Furthermore, it was found that bare soil destroyed about 75% more ozone when dry than when moist. The determination of ozone flux to surfaces may be a far more realistic measure of dose to living organisms than atmospheric concentration of ozone, according to Munn.[46]

Indirect Effects

The concentration of plant nutrients in litter influences both the rate of decomposition and the amount of nutrients released after decomposition. Ozone-injured foliage may be deficient in inorganic nutrients, because of the concomitant decay of the root systems of chronically injured trees.[54]

Coniferous leaf litter is more resistant to decomposition than broad-leaved litter.[41] Both low inorganic nutrient status and the presence of organic compounds that are toxic to microorganisms may account for slower decay. Ozone injury to needles or broad leaves might result in an accumulation of phenolic compounds that are toxic to bacteria and fungi. The rate of litter decomposition might be decreased. A high carbon:nitrogen ratio related to nitrogen deficiency may also slow decomposition.

Katz and Lieth[31] have categorized the roles of both microflora and microfauna groups in litter decomposition. They have discussed the successional trends of microflora and microfauna populations during the warm season. A typical problem emerges when population sampling is attempted: "a consistent feature of communities is that they contain a comparatively large number of species that are rare at any given locus in time and space."[49] The task of identifying interactions (see Figures 12-9 through 12-12) of decomposer populations with pollutants, particularly oxidants, is formidable.

In the San Bernardino Mountains, studies are going on to describe the effects of oxidant injury to ponderosa and Jeffrey pines on the microarthropods and fungi of the litter layer under trees with various degrees of

injury. Initial observations suggested lower population densities of microarthropods in the classes Insecta, Arachnida, and Myriapoda under some severely injured trees.[31a]

A coincident effect on decomposers may be from the accumulation of heavy metals, such as lead, which is entrained in the photochemical oxidant complex. In Sweden, Ruhling and Tyler[59] have preliminary evidence that litter decomposition rate in a spruce forest was limited by increased concentration of heavy-metal ions, but only during times of the year when water and temperature were not limiting factors.

The difficulties of understanding population changes of litter-decomposing microflora and microfauna as an index of pollutant effects suggest that a more simple approach should be used at the outset. The least sophisticated expression of accepted procedure[51] is:

$$\frac{\Delta x}{\Delta t} = \text{income for interval} - \text{loss for interval},$$

where Δx is the change in amount of litter over the interval t. This expression describes energy storage in litter and indicates the balance between producers and decomposers. Typical annual litter production, steady-state accumulation on the forest floor, and associated decomposition rate factors have been estimated for several locations and types of forest litter.[51] Comparisons of litter decomposition rate factors under similar stands of trees subjected to various doses of oxidant air pollutants could be useful for quantifying the dynamics of ozone-induced tree decline. This approach is being investigated by Arkeley (in Kickert et al.[31a]) in the San Bernardino Mountains.

Sophisticated decomposition models are being developed. A simulation model developed by Bunnell and Dowding[8] for tundra sites is a nine-compartment model with 23 transfers between compartments. This type of model may provide the only method for understanding the extremely complex litter decomposition process.

In summary, it is anticipated that decreasing litter production by green plants experiencing pollutant stress would result in a similar reduction in the inventory of nutrient elements held within the system, owing to the interruption of cycling pathways and mechanisms of nutrient conservation.[37]

CONCLUSIONS

The transport of injurious concentrations of ozone and other oxidants to rural areas downwind from urban centers at numerous locations in the United States appears to be on the increase.[11, 31a] Blumenthal *et al.*[5] conservatively estimate that the urban plume from the Los Angeles area "could cause ozone concentrations to exceed the Federal standard of 0.08 ppm at locations as far as 260 km." Other areas where significant rural concentrations of oxidant have been observed are Salt Lake City, Denver, and the Blue Ridge Mountains.

In general, the permanent vegetation constituting natural ecosystems receives much greater chronic exposure, and the short-lived higher-value vegetation constituting the agroecosystem of the Los Angeles coastal plain can be subject to injurious doses, but in intermittent short-term fumigations. Each situation has measurable economic and aesthetic effects, but on different time-scales. The single agricultural ecologic system (the agroecosystem) has little resilience to pollutant stress; losses are sometimes immediate and occasionally catastrophic. The complex natural ecosystem is initially more resistant to pollutant stress, but the longer chronic exposures cause disruption of both structure and function in the system that may be irreversible.

Simulation models of ecosystem subsystems are developing rapidly.[47, 52] They deal with flows of energy, biomass, mineral nutrients, water, numbers of species, population densities, and area occupied per biotic unit. Interactions between ecosystem components must be understood before prediction can be attempted. Simulation models offer a bright opportunity for determining

the long-term effects on natural ecosystems and agroecosystems. New knowledge of biologic effects should suggest the importance of prevention and some means for ameliorating damage.

Oxidant injury to the mixed-conifer stands of the San Bernardino Mountains beginning in the early 1940's is well advanced. A similar problem is developing in the forests of the southern Sierra Nevada. Both places show both direct and indirect effects on all subsystems of the forest ecosystem -- producers, consumers, and decomposers. For example:

- Ozone injury limits biomass production by the primary producers and their capacity to reproduce.
- The decrease of biomass or energy flow to consumer and decomposer in the ecosystem affects the populations of these organisms.
- Essential recycling processes, such as recycling of nutrients, may be interrupted, further limiting primary production.
- Stand structure is altered rapidly in some areas by salvage logging of high-risk trees; as a result, species composition is changing, and wildlife habitat is being altered.

Oxidant injury to eastern white pine in some forest stands in the eastern United States is a significant problem. There is an important concern about injury caused by a synergistic reaction between ozone and sulfur dioxide at low concentrations.

The relationship between man's welfare and stable natural ecosystems and agroecosystems can be established in terms of the economic and aesthetic values derived from them. In some situations, where ecosystems are stressed by oxidant pollutants, the benefits realized by present and future generations may soon diminish. Considerable research is required to find alternatives that prevent stress or that may salvage some of these benefits. New management

strategies should be instituted only when their consequences are predictable within reasonable limits.

RECOMMENDATIONS

The present knowledge of the biologic consequences of chronic oxidant injury to both natural ecosystems and agroecosystems must be communicated to groups in the public sector that work in planning and enforcement. The indirect effects on man's health and the direct effects on his welfare resulting from ecosystem deterioration due to oxidant injury are serious enough to be given more thorough consideration in all decisions related to abatement of air pollution from both mobile and stationary sources. Land-use planning and proper airshed classification should be used to prevent further deterioration of air quality, particularly in prime timber-producing areas and in remote, pristine areas, regardless of their present use designation.

The most important research needs are related to the determination of the responses of natural ecosystems and agroecosystems to chronic exposure to oxidant pollutants. In particular, chronic-dose-response models are needed to understand the responses of the dominant primary-producer species constituting forest ecosystems in both the eastern and the western United States. The resulting alteration of interactions with other subsystems--e.g., consumers and decomposers--must also be investigated.

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Chapter 13

EFFECTS OF PHOTOCHEMICAL OXIDANTS ON MATERIALS

The effect of photochemical oxidants on materials has been investigated by exposure of materials to ambient air containing photochemical oxidants, including ozone, PAN, peroxybenzoylnitrate (PBzN), and all other molecules in ambient air that oxidize iodide ion (but not nitrogen dioxide), as prescribed in the photochemical oxidant ambient air quality standard.[1] Laboratory experiments for corroboration of field tests always use ozonized air that contains no NO_x if photochemical generation is used and may contain NO_x if electric discharge is the method of ozone generation. The reactivity of oxidizing species, such as atomic oxygen and excited electronic states of molecular oxygen, must be assessed both in test chambers and in ambient air before blame for damage is assigned to ozone. On the presumption that ozone is the damaging species in test-chamber experiments in which PAN and similar oxidants are absent, photochemical oxidant damage is often referred to in this discussion as "ozone" damage. The subject has been reviewed in Air Quality Criteria for Photochemical Oxidants[2] and in a systems study by Salmon[3] and more recently by Sanderson.[4] Other, rather comprehensive, although specialized, reviews have appeared on the effects of air pollutants on textiles and dyes,[5] on rubber,[6] and on paint[7,8] and on their economic effects.[9] In preparing this document, the literature has been reviewed with special emphasis on papers published since 1970, when the EPA criteria document appeared.[2]

TEST-CHAMBER STUDIES

Experimental studies on the effects of ozone on materials usually involve the laboratory generation[10] of ozone by photolysis of air with a mercury resonance lamp or by some form of electric discharge through air or oxygen. Several oxidizing species result, among which are the first singlet state of atomic oxygen, $O(^1D)$; ground-state atomic oxygen, $O(^3P)$; the lowest-lying singlet molecular oxygen, $O_2(^1\Delta_g)$; and ozone. To identify the species responsible for the effect, one must know the rate constants characteristic of the interactions of these species with the material in question and the relative concentrations of the species at the contact surface between the ozonized air and the damaged material.

The Photochemical Ozone Generator

The standard ASTM test method[10] (D-1149-64) for rubber damage includes a test chamber (volume, 0.11-0.14 m^3) through which ozonized air flows at a rate greater than 0.6 m/s. Because the residence time of the ozonized air in the test chamber is about 1 s, the ozone may be expected to reach the material in about 0.1 s. A somewhat similar test procedure[10] (AATCC test method 109-1972; ANSI L14, 174-1973) is used in testing colorfastness. The ozone generator is usually (but not necessarily) a mercury-vapor resonance lamp with emission lines at 184.9 and 253.7 nm. The 184.9-nm line is absorbed, and two ground-state oxygen atoms are produced:

$$O_2 + h\nu\ (184.9\ nm) \rightarrow 2O(^3P). \qquad (1)$$

Each oxygen atom then reacts with diatomic oxygen to form ozone:

$$O + O_2 + M \rightarrow O_3 + M, \qquad (2)$$

where M signifies any gaseous molecule, such as O_2 or N_2. Usually, the 253.7-nm line is more intense than the 184.9-nm line and falls in a very strong absorption band of ozone:

$$O_3 + h\nu \quad (253.7\ nm) \rightarrow O(^1D) + O_2(^1\Delta_g). \tag{3}$$

The species produced by this photochemical reaction can be quenched to their ground electronic states by collisional processes:

$$O(^1D) + M \rightarrow O(^3P) + M; \tag{4}$$

$$O_2(^1\Delta_g) + M \rightarrow O_2(^3\Sigma_g^-) + M. \tag{5}$$

In addition, the ground-state oxygen atom reacts with ozone:

$$O + O_3 \rightarrow 2O_2. \tag{6}$$

Rate constants of these reactions are summarized in Table 13-1.

Concentrations in the Generator. The absorbed intensity, I_a, is obtained from the Beer-Lambert law, which relates the incident intensity, I_o, and the transmitted intensity, I_t,

$$I_t/I_o = \exp(-\varepsilon c \ell), \tag{7}$$

where ε is the absorption coefficient, c is the concentration of the absorbing species, and ℓ is the path length. It follows that

$$\frac{I_o - I_t}{I_o} = \frac{I_a}{I_o} = 1 - \exp(-\varepsilon c \ell). \tag{8}$$

Table 13-1

Rate Constants at a Temperature of 300 K(M ≡ O_2 or Air)

Reaction	Rate Constant (k)	Reference
(1) $O_2 + h\nu$ (184.9 nm) → 2O	$\varepsilon_1 = 2.0 \times 10^{-20}$ molec^{-1} cm^2 (base e)	11
(2) $O + O_2 + M \rightarrow O_3 + M$	6.0×10^{-34} molec^{-2} cm^6 s^{-1}	12
(3) $O_3 + h\nu$ (253.7 nm) → $O(^1D) + O_2(^1\Delta_g)$	$\varepsilon_3 = 1.27 \times 10^{-17}$ molec^{-1} cm^2 (base e)	13
(4) $O(^1D) + M \rightarrow O(^3P) + M$	7.5×10^{-11} molec^{-1} cm^3 s^{-1}	12
(5) $O_2(^1\Delta_g) + O_2(^3\Sigma_g^-) \rightarrow 2O_2(^3\Sigma_g^-)$	2.2×10^{-18} molec^{-1} cm^3 s^{-1}	12
(6) $O + O_3 \rightarrow 2O_2$	8.6×10^{-15} molec^{-1} cm^3 s^{-1}	12

For $\varepsilon c\ell \ll 1$,

$$I_a = I^o \varepsilon c \ell . \quad (9)$$

The stationary-state expressions are:

$$d[O]/dt = 2I_1 - k_2 [O][O_2][M] + [O(^1D)][M] - k_6 [O][O_3] = 0; \quad (10)$$

$$d[O(^1D)]/dt = I_3 - k_4 [O(^1D)][M] = 0; \quad (11)$$

$$d[O_3]/dt = k_2 [O][O_2][M] - I_3 - k_6 [O][O_3] = 0; \quad (12)$$

The values of I_1 and I_3 --the absorbed light intensities at 184.9 nm and 253.7 nm, respectively--are related to incident intensities by expressions like Eq. 8. The incident intensities depend heavily on lamp design and construction. For the purpose of the stationary-state computation, we use (on the basis of experience) $I_1 = 10^{17}$ photons/cm^3 and $I_3 = 3 \times 10^{17}$ photons/cm^3-s. In the calculations, a 1-cm path length is assumed. Summing Eqs. 10-12 and referring to Table 13-1,

$$I_1 = k_6 [O][O_3] = I_1^o \varepsilon_2 [O_2]. \quad (13)$$

The oxygen concentration in air is taken to be 5×10^{18} molecules/cm^3.

$$[O] = 1.16 \times 10^{30} / [O_3]. \quad (14)$$

The concentration of $O(^1D)$ is obtained from Eq. 11.

$$[O(^1D)] = \frac{I_3}{k_4 [M]} = \frac{I_3^o \varepsilon_3 [O_3]}{k_4 [M]} = 2.1 \times 10^{-9} [O_3]. \quad (15)$$

The concentration of $O_2(^1\Delta_g)$ is obtained by means of its stationary-state expression:

$$\frac{d[O_2(^1\Delta)]}{dt} = I_3 - k_5[O_2(^1\Delta)]M = 0; \tag{16}$$

$$[O_2(^1\Delta)] = \frac{I_3 \varepsilon_3^o [O_3]}{k_5 M} = 7.1 \times 10^{-2} [O_3]. \tag{17}$$

Because $I_1 = k_6[O][O_3]$ and $I_3 = k_4[O(^1D)][M]$, Eq. 10 yields:

$$I_1 + I_3 = k_2[O][O_2][M]. \tag{18}$$

Substituting for [O] and solving with the quadratic formula:

$$I_1 + I_3 = k_2[O_2][M] \times 1.16 \times 10^{30} = I_1 \varepsilon_1^o [O_2] + I_3 \varepsilon_3^o [O_3]; \tag{19}$$

$$\underbrace{I_3 \varepsilon_3^o}_{a} [O_3]^2 + \underbrace{I_1 \varepsilon_1^o [O_2]}_{b} [O_3] \underbrace{- k_2[O_2][M] \times 1.16 \times 10^{30} = 0}_{c} \tag{20}$$

$$[O_3] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \tag{21}$$

Evaluating a, b, and c:

$a = 3.3$,

$b = 3.7 \times 10^{15}$, and

$c = 8.5 \times 10^{34}$,

the solution is $[O_3] = 1.6 \times 10^{17}$ molecules/cm^3. The concentrations of the oxidizing species for $[O_3] = 1.6 \times 10^{17}$ molecules/cm^3 are given in Table 13-2. The ratio of rates of attack of $O(^3P)$ and ozone on a material in the ozone generator is given by:

$$R_O/R_{O_3} = \frac{[O(^3P)]}{[O_3]} \cdot \frac{k_O}{k_{O_3}}, \quad (22)$$

where k_O and k_{O_3} are the rate constants. A hypothetical example of a material undergoing a damage test may be chosen to be an olefinic polymer. In the absence of data on appropriate material, we may presume that damage done to an olefinic material by oxygen atoms and ozone would proceed at the same relative rates as attack on a typical olefin, e.g., butene-2. Rate constants for attack of various oxygen species on *t*-butene-2 are presented in Table 13-3. Thus, we estimate

$$k_O/k_{O_3} = 3.5 \times 10^5 \quad (23)$$

and $$R_O/R_{O_3} = (4.5 \times 10^{-5})(3.5 \times 10^5) = 15.8. \quad (24)$$

It follows that, although the ozone concentration is more than 10,000 times that of the oxygen atom, the latter reacts so rapidly with olefins that oxygen atoms, rather than ozone, will be the species responsible for material damage, if the material is placed in the ozone generator.

Table 13-2

Stationary State Concentrations of Oxidants in Photochemical Ozone Generator[a]

Oxidant	Concentration, $molecules/cm^3$
O_3	1.6×10^{17}
$O(^3P)$	7.3×10^{12}
$O(^1D)$	3.4×10^{8}
$O_2(^1\Delta_g)$	1.1×10^{16}

---

[a] $\ell = 1$ cm, $I_1^o = 10^{17}$ photons/cm^3-s, $I_3^o = 3 \times 10^{17}$ photons/cm^3-s.

Table 13-3

Rate Constants for Reaction of Oxygen Species with trans-2-Butene[a]

Oxidant	Rate Constant (k, molec^{-1} cm^3 s^{-1})
$O_2(^1\Delta_g)$	4.6×10^{-18}
O_3	3.5×10^{-17}
$O(^3P)$	2.3×10^{-11}

[a] Data from Demerjian et al.[18]

Similarly, for $O(^1\Delta_g)$:

$$\frac{R\,O(^1\Delta_g)}{R\,O_3} = \frac{k_{\Delta}}{k_0} \cdot \frac{[O(^1\Delta)]}{[O_3]} = \frac{2.3 \times 10^{-18}}{1.1 \times 10^{-17}} \cdot \frac{1.1 \times 10^{16}}{1.6 \times 10^{17}} = 0.014. \quad (25)$$

Therefore, $O(^1\Delta_g)$ could be a significant problem in ozone damage tests on olefin-like materials, if the test were done in the ozone generator. The results in Table 13-2, although only very approximate because of the assumptions made, show that in a photochemical ozone generator we may expect $O(^1\Delta_g)$ concentrations to be an appreciable fraction of the ozone concentration. The $O(^1D)$ concentration is negligibly small, because it is removed in the reaction shown in Eq. 4 on almost every collision.

Concentration Downstream from the Generator. The ASTM method requires that the ozone generator be outside the test chamber, and we must ask how long $O(^1\Delta_g)$ and $O(^3P)$ survive after the ozonized air leaves the generator on its way to the material under test. About 0.1 s is required for the ozone to reach the material after leaving the generator. As the light source is extinguished (t = 0) or as the ozonized air leaves the photolysis chamber, the rate at which $O(^1\Delta_g)$ is removed is--using S to signify $O(^1\Delta_g)$--

$$-\frac{d[S]}{dt} = k_5[S][M]; \quad (26)$$

$$-d\ell n[S] = k_5[M]dt. \quad (27)$$

Thus, 0.1 s after the light is turned off, $[S_o]$ decays to [S] by Eq. 5, whose rate constant is given in Table 13-1. The concentration, [M], is 2.7×10^{19} molecules/cm^3. Solving Eq. 27, we obtain

$$-\int_{[S]_o}^{[S]} d\ell nS = 6; \tag{28}$$

$$\ell n \frac{[S]}{[S]_o} = -6; \tag{29}$$

$$\frac{[S]}{[S]_o} = 10^{-2.6}. \tag{30}$$

Therefore, although the $O_2(^1\Delta_g)$ is only very inefficiently quenched by collision, at a pressure of 1 atm quenching is sufficiently rapid that only a few tenths of 1% of the original $O_2(^1\Delta_g)$ can reach the test material. However, when the pressure is smaller--say, 76 torr or 2.5×10^{18} molecules/cm^3 --$\ln([S]/[S]_o) = -0.6$ after 0.1 s and more than half the original $O_2(^1\Delta_g)$ molecules may reach the test material.

Finally, we must ask whether the ASTM procedure is likely to result in removal of oxygen atoms sufficiently rapid for the observed damage to be attributed to ozone. The rate of removal of oxygen atoms by the reaction of Eq. 2, which is the major sink, is given by:

$$-d[O]/dt = k_2 [O][O_2][M]; \tag{31}$$

$$-\int_{[O]_o}^{[O]} d\ell n[O] = k_2 [O_2][M] \int_0^{0.1} dt; \tag{32}$$

$$\ell n \frac{[O]}{[O]_o} = -8.1 \times 10^{3}; \tag{33}$$

$$[O]/[O]_o = 10^{-3500}. \tag{34}$$

Therefore, the ASTM test is a valid ozone damage test, inasmuch as both the oxygen atoms and the $O_2(^1\Delta_g)$ are rapidly removed downstream from the generator. The importance of having the ozone generator outside the test chamber cannot be overemphasized. The validity of any ozone damage test using other sources depends very heavily on the test procedure. If an electric discharge is used as the ozone generator, the test chamber should be designed in accord with such chemical kinetic considerations as indicated in the model discussed here. The relative concentrations of oxidizing species in a radiofrequency or microwave discharge or a photochemical generator are subject to conditions and to some speculation. However, equal concentrations of $O(^3P)$ and $O(^1\Delta_g)$ have been suggested as emerging from discharged air.[17] The ratio of oxygen atoms to ozone depends critically on the pressure. In a microwave discharge where the pressure may be 1 torr, 0.1 s after leaving the discharge a substantial fraction of the oxygen atoms remains.

Because oxygen atom reaction rate constants can be orders of magnitude greater than those for ozone, an experiment done on material subject to a reduced-pressure discharge is likely to signify damage done by oxygen atoms, rather than ozone.

Oxidant Species Concentrations in Polluted Air

We have shown that, in a properly designed laboratory experiment, ozone is likely to be the only oxidant species producing damage in the test material. In a real atmosphere, such photochemical oxidants as PAN and PBzN are formed in complex atmospheric reactions. Because there is no information on the effects of these oxidants on materials, no consideration is given to them in assessing material damage in this discussion. Sunlight and energy transfer processes result in generation of $O(^1\Delta_g)$ and particularly $O(^3P)$. Therefore, it is necessary to consider the concentrations of these species relative to that of ozone in a real atmosphere. This has been done, notably by Demerjian *et al.*,[18] one of whose estimates, calculated from smog-chamber considerations, is shown in Table 13-4. The ozone concentration assumed in Table 13-4 is typical of a fairly polluted atmosphere. To estimate the relative contributions of each oxidizing species to oxidant damage, it is necessary to multiply the concentration by the rate contant for each individual material. Unfortunately, there is almost no information on rate constants, and it is assumed in nearly all cases that the oxidizing species is ozone.

EFFECTS OF OXIDANTS ON INDIVIDUAL MATERIALS

According to the Midwest Research Institute study of 1970,[3] both ferrous and nonferrous metals have excellent resistance to ozone. Such materials as building stone, building brick, cement, glass, and graphite also have excellent resistance. Some synthetic rubbers have good ozone resistance, but natural rubber has notoriously poor resistance. Polyethylene, polystyrene, and polypropylene are believed to have only fair resistance to ozone, and

Table 13-4

Calculated Concentrations of Oxidant Species in Smog Chambers[a]

Oxidant	Concentration, molecules/cm^3
$O_2(^1\Delta_g)$	1.4×10^8
O_3	3.8×10^{12}
$O(^3P)$	2.6×10^5
OH	1.9×10^6
HO_2	1.0×10^{10}

[a] Calculated concentrations for oxidants in smog chamber under conditions outlined in Chapter 2 for 60-min irradiation (see Table 2-2).

acetate, nylon, and fibers of cotton, rayon, and cellulose esters are also in the "fair" category. Wood is considered fairly resistant, but little information is available. The approach used by MRI in assessing economic effects is that the value of material exposed to air pollution, Q, is the product of four factors: the annual production, P; the average economic life of the material, N; the fraction of material exposed to air pollution, F; and the labor factor, R, which reflects the cost of putting the material in place where it is used. Hence,

$$Q = P \cdot N \cdot F \cdot R. \tag{35}$$

Having defined Q, the MRI report defines the damage costs as the product of Q and ν, the "interaction" value per year. For example, if Q = \$1 billion and a pollutant causes a 1% reduction in economic life per year of exposure, then $\nu = 0.01$, and the economic loss, L, due to the pollutant is $Q\nu$, or \$10 million/year. The values of ν for pollutant damage generally are difficult to obtain, because few data are available. For the particular pollutant, ozone, the values of ν are almost impossible to assess. The MRI report points out that "intuitive feelings" are sometimes used to assess ν. The materials that have been classified poor or fair in ozone resistance are listed in Table 13-5 in order of decreasing in-place value. The chemical interaction value, ν, and the economic loss, L, are also tabulated. Because it is difficult at best to estimate overall pollutant interaction, ozone damage has been assessed in the last column of Table 13-5 by estimating L', the ozone damage, as follows: If chemical resistance has been estimated by MRI as either poor or fair for one pollutant other than ozone for which a

Table 13-5

Economic Value of Ozone-Sensitive Materials Exposed to Pollution[a]

Material	P, $Million	N, years	F	R	Q, $Million	ν, per year	L, $Million	L', $Million
Paint	2,587	4	0.7	3.3	23,900	0.05	1,195	600
Wood	10,875	30	0.03	1.8	17,620	0.001	18	9
Cotton	3,336	6	0.1	1.9	3,800	0.04	152	76
Polyethylene	975	6	0.2	1.0	1,170	0.01	12	12
Nylon fiber	830	6	0.1	1.9	950	0.04	38	13
Polystyrene	850	5	0.2	1.0	850	0.01	9	9
Polypropylene	403	8	0.2	1.0	640	0.01	6	6
Natural rubber	1,790	6	0.05	1.0	540	0.10	54	27
Rayon fiber	288	6	0.1	1.9	330	0.04	13	7
Nylon plastics	106	8	0.2	1.0	170	0.01	2	1
Acetate plastic	58	10	0.2	1.0	120	0.01	1	0.5

a L' refers to ozone damage; ν and L refer to total gaseous pollutant damage. See text for explanation of column headings.

national ambient air quality standard has been set, $L' = \frac{1}{2}L$; if two other pollutants are in the poor or fair category, $L' = 1/3L$. The data in Table 13-5 should be regarded as very rough and indicative only of broad-brush outlines. For example, paint damage is undoubtedly a very important economic consequence of ozone pollution, but, as we shall see, the estimate of rubber damage in Table 13-5 is much too low.

The most studied materials, as well as the most important economically, with respect to ozone damage are paint and elastomers (e.g., rubber). Other materials will be reviewed individually in cases in which scientific data are available.

Paint

Failure of Paint Films. Paint formulation consists of a binder (a natural or synthetic polymer or drying oil), a solvent, and a pigment or colorant, including an extender, typically calcium carbonate or a silicate. Because of the reactivity of organic polymers toward ozone, it is not surprising that ozone damage has been observed, at least in laboratory experiments. In 1968, paint sales of over $2.5 billion were recorded in the United States. Table 13-6 outlines these sales.[8] The failure of paint films takes on a variety of forms, as shown in Table 13-7, and methods for testing the various forms of failure have been delineated by the ASTM.[19] These methods are listed in Table 13-8. The paint industry has been concerned mainly with "weatherability" of paints, rather than specifically with the effect of air pollutants.

Table 13-6

Trade and Industrial Paint Sales, United States, 1968[a]

Type of Sale	Amount, millions of gallons	Cost, $ millions	Unit Cost, $/gal
Trade:	424	1,428	3.37
Interior house paints:			
Latex emulsion	145	425	2.93
Oil and alkyd	45	170	3.78
Primer, sealers, etc.	10	30	3.00
Miscellaneous	25	100	4.00
Exterior house paints:			
Latex emulsion	55	190	3.45
Oil and alkyd	40	155	3.88
Enamels	15	55	3.67
Primers, sealers, etc.	10	35	3.50
Miscellaneous	20	50	2.50
Automotive refinishing	30	150	5.00
Traffic	25	45	1.80
Other	4	23	5.75
Industrial:	419	1,159	2.77
Automotive, new	55	170	3.09
Marine	20	85	4.25
Railroad, aircraft, etc.	15	40	2.67
Coil coating	20	80	4.00
Prefinished wood	15	40	2.67
Industrial maintenance	45	160	3.56
Machinery and equipment	30	75	2.50
Miscellaneous	219	509	2.32

[a] Data from Spence and Haynie.[8]

Table 13-7

Types of Film Failure[a]

Type Failure	Description
Chalking	Formation of powdery layer on surface of coating that is being eroded away
Cracking or checking	Shrinkage of the coating resulting in film rupture
Alligatoring	Film rupture resulting from the application of a brittle film over a more flexible coating
Peeling	Poor adhesion of the coat to substrate
Color fading	Reaction of binder or pigment in presence of sunlight or environment
Blistering	Projections or pimples on film that result from trapping of solvent or moisture between substrate and film
Rusting	Oxidation of metallic substrates, such as iron or steel, when film permits moisture or chemicals to attack substrates

[a] Data from Spence and Haynie.[8]

Table 13-8

ASTM Test Methods for Exterior Paint Films

Test	Purpose
D 659-44	Evaluating degree of resistance to chalking
D 660-44	Evaluating degree of resistance to checking
D 661-44	Evaluating degree of resistance to cracking
D 662-44	Evaluating degree of resistance to erosion
D 772-47	Evaluating degree of resistance to flaking (scaling)
D 1641-59	Measuring exterior durability of varnishes
D 1543-63	Measuring color change of white architectural enamels
D 1654-61	Evaluating painted or coated specimens subjected to corrosive environments
D 2197-68	Measuring adhesion of organic coatings
D 2370-68	Measuring elongation and tensile strength of free films of paint, varnish, lacquer, and related products with tensile testing apparatus

Probably the most definitive study of the effect of air pollutants on paint is contained in a 1972 report of the Sherwin-Williams Company.[20] Five commercially important coating systems were selected for study:

1. House paint--lead-titanium-zinc extender in oil with 100% rutile titanium dioxide.
2. House paint--titanium extender in acrylic latex with 100% rutile titanium dioxide.
3. Industrial maintenance coatings--titanium in alkyl with 100% rutile titanium dioxide.
4. Coil coating finishes--titanium extender in urea-alkyl with 75% rutile and 25% anatase titanium dioxide.
5. Automotive refinish lacquer--titanium in nitrocellulose-acrylic with 100% rutile titanium dioxide.

For each coating system, three tasks were performed:

- Existing exterior exposure records were reviewed with respect to visual erosion ratios.
- Short-term exposures were studied at Leeds, North Dakota; Los Angeles, California; Chicago, Illinois; and Valparaiso, Indiana. The North Dakota location was a clean, rural site; Los Angeles represented a high-oxidant urban site; Chicago was a high-sulfur dioxide location; and Valparaiso was considered to have moderate sulfur dioxide pollution and low oxidant concentrations. Tests with commercial instruments included those for erosion, gloss, sheen, surface roughness, and tensile strength.

- Accelerated laboratory exposures were made at various pollutant concentrations: no pollutant, 0.1-ppm sulfur dioxide, 1-ppm sulfur dioxide, 0.1-ppm ozone, and 1-ppm ozone.

As measured by erosion rates (linearly related to pollutant concentration), it was found that in all cases coatings were affected more by sulfur dioxide than by ozone. Sulfur dioxide at 1 ppm had the following effects on the five paint systems: System 1, "considerable"; systems 2 and 4, "moderate"; and systems 3 and 5, "no effect." Both attenuated total reflection and scanning electron photomicrographs showed that the damage was greater in Chicago and Valparaiso than in Los Angeles and North Dakota.

Studies have been conducted on creep compliance tests[8] in which paint films were subjected to tensile loads of 4-7 $lb/in.^2$ ($27.2\text{-}47.6 \times 10^3$ N/m^2) and to 6% ozone for 505 h. A typical result for a high-quality emulsion-base paint is shown in Figure 13-1. Creep compliance is reduced by exposure to 6% ozone. If the effect is linearly related to ozone concentration, we might expect the same reduction in creep compliance at 0.1-ppm ozone in 3×10^8 h, or some 30,000 years. Thus, reduction in creep compliance is not viewed as having a serious ozone contribution.

Economic Effects. The Midwest Research Institute Report (pp. 41 ff.) computes L, the increased cost of cleaning or repainting per year, as the product of Q, the consumer purchase price of a unit of material; γ, the cost of cleaning or repainting one such unit; and Δf, the fractional increase in cleaning frequency resulting from particulate pollution.

$$L = Q \cdot \gamma \cdot \Delta f, \text{ dollars/(yr. x unit)}. \tag{36}$$

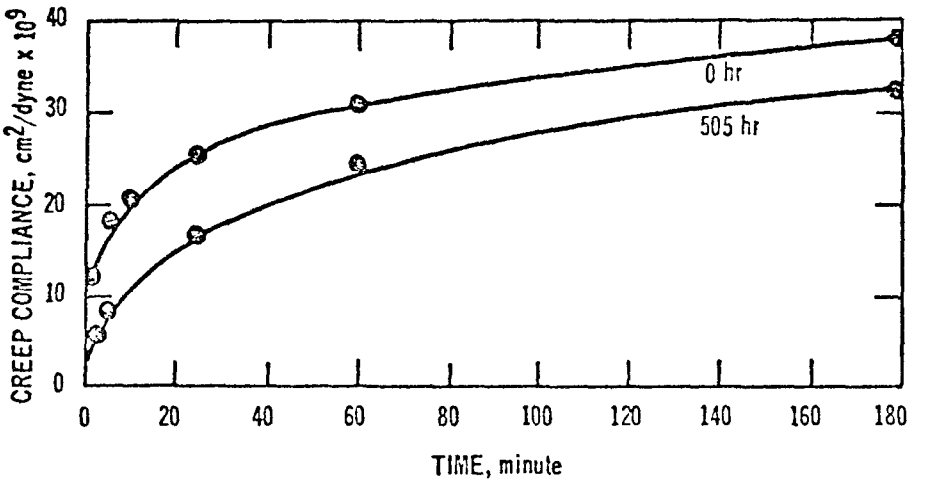


Figure 13-1. Initial creep of EM 7338 first-quality emulsion-base paint, before and after 505-h exposure to ozone at 60 C. (Reprinted from Spence and Haynie.[8])

The cost, γ, of cleaning one unit is determined for two classes of material, fibers and nonfibers. For fibers, \$0.10/lb is taken as the cost of cleaning; for nonfibers, \$0.10/ft^2 is taken as the cost of cleaning (removal of particulate material). The purchase price is defined as the material cost times a factor that gives the consumer purchase price including labor (painters, outlet salesmen and their overheads, etc.). The quantity $\gamma\Delta f$ is given by $\nu = \gamma\Delta f = L/Q\text{-yr}$ and is called the soiling interaction:

$$L = Q\nu. \tag{37}$$

The determination of the quantity Δf, the fractional increase in cleaning frequency due to particulate matter, is the difficult part of the problem. The data of Michelson and Tourin[21] suggest a value of ν for paint of 1.5/year. The value of Q is obtained as follows: According to Noble,[22] the annual production of paint in 1968 was \$2.59 billion. Because the economic life of paint is considered to be 4 years, \$10.36 billion worth of paint is in place, of which only 70% is exposed to atmospheric pollutants. Thus, \$7.25 billion is the manufacturers' value of in-place paint exposed to air pollution. Using a labor factor of 3.3, the total in-place value of paint is \$23.9 billion. Because $\nu = 1.5$ for particulate soiling, the cost of pollution damage (cleaning costs) is some \$36 billion per year.

The costs associated with chemical effects of gaseous pollutants could not be ascertained for each pollutant, because of a paucity of information. The value of ν for chemical deterioration was obtained by an educated guess that repainting is needed every 4 years in a polluted environment and every 5 years in a pristine air environment. Thus, ν (chemical) = 0.05. The

corresponding figure for chemical deterioration costs is 0.05 ($23.9 billion) = $1.195 billion per year.

Spence and Haynie have attempted to assess costs of air pollution damage to paints without special regard to the identity of the pollutant doing the damage. However, repainting frequencies have been determined in five American cities whose particulate concentrations have been measured. There appears to be a linear (although not necessarily causal) relationship between the maintenance frequency and particulate concentration.[21] Economic loss due to pollution was estimated by the following procedure: The average service life of exterior household paint in urban areas is 3 years, and in rural areas, 6 years. Thus, 0.33 and 0.17 are the replacement probabilities per year. Because 60% of the surface painted is urban and 40% rural, it may be calculated that some 74% of the paint used over a long period is consumed in urban areas. The total value of manufactured paint is $485 million, of which 74%, or $359 million per year, is the cost of paint consumed in urban areas. Its service life is 50% less than in rural areas, so 0.50 ($359 million) = $180 million is the annual cost of exterior household paint deterioration due to pollution. The value of loss of paint in place was computed to be 3.0 ($180 million) = $540 million. It is noteworthy that the Midwest Research Institute report estimates $1.195 billion repainting costs due to chemical pollution.

The economic assessment of air pollution damage to exterior paints is summarized in Table 13-9. There appears to be no definitive way to determine the fraction of the $225 million loss for all exterior paints that is due to photochemical oxidants. According to the convention introduced by Midwest

Table 13-9

Economic Assessment of Air Pollution Deterioration of Exterior Paints (1968 Figures--Manufacturers Costs)[a]

Exterior Paint Class	Value of Paint Exposed in Urban Areas, $ million	Loss Due to Air Pollution, $ million
Coil coating[b]	31	8
Automotive refinishing	111	22
Industrial maintenance	74	15
Household[b]	359	180
Total	575	225

[a] Data from Spence and Haynie.[8]

[b] Extender Type Paints.

Research Institute--that repainting costs may be considered to be due to chemical pollution--only NO_x, sulfur dioxide, and photochemical oxidants seem likely to be involved in this kind of paint damage. Sulfur dioxide seems to attack only the extender (e.g., calcium carbonate) component. It might also be expected that nitrogen dioxide would attack only extender paints. Sulfur dioxide has no effect on the nonextender types--industrial maintenance and automotive refinishing paints. Therefore, the $37 million worth of damage done to the nonextender types each year may be attributed to the effects of oxidants. Of the $188 million worth of damage to extender paints, we may expect some 80% to be caused by sulfur dioxide and nitrogen dioxide, with $38 million worth of damage (20%) due to oxidants. Thus, a total of $75 million worth of paint damage per year is caused by oxidants. The figure of $75 million includes only the cost at the manufacturers' level. The cost at the consumer level--i.e., the total cost of the paint applied to a surface--is obtained by multiplying by a factor of 3.0 (according to Spence and Haynie[8]) or 3.3 (according to Salmon[3]). Thus, the ozone damage to paint (using a 3.3 factor) is $248 million per year.

Elastomers

Failure of Elastomers. Elastomers include both synthetic elastomers and natural rubber. The most extensive study of the effects of ozone on elastomers (including economic effects) was done by Mueller and Stickney[6] and Stickney *et al.*,[23] and much of what follows is taken from the 1971 report.[23] Table 13-10 presents the estimated elastomer production in 1975. The effect of ozone on cracking of natural rubber was proved by Newton[24] and by Crabtree and Kemp.[25] In general, highly unsaturated elastomers are

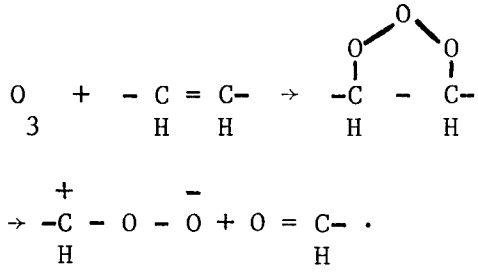
Table 13-10

Polymer Usage Projected to 1975[a]

Polymer	Estimated Production, 1,000 tons[b]	Estimated Average Price Per Pound, cents[c]	Value, $ million	Portion of Total Polymer Value, %
SBR--copolymer of butadiene and styrene	1,748	23	804.1	39.8
Natural--natural polyisoprene	689	22	303.2	15.0
Polybutadiene--polymerized butadiene	375	27	202.5	10.0
Neoprene--polymerized chloroprine	146	40	116.8	5.8
Nitrile--copolymer of butadiene and acrylonitrile	95	50	95.0	4.7
Urethane--polymers containing urethane linkage	45	125	112.5	5.6
Butyl--copolymer of isobutylene and isoprene	101	27.5	55.5	2.7
Silicone--polydimethylsiloxane	13	250	65.0	3.2
Polyisoprene--synthetic polyisoprene	134	26	69.7	3.4
Polyacrylate--polymer or copolymer based on an acrylate	3	80	4.8	0.2
EPDM--copolymer of ethylene, propylene, and a diene	168	25	84.0	4.2
Polysulfide--polysulfide	17	135	45.9	2.3
Fluorocarbon--fluorinated polymers	2	1,250	50.0	2.5
Chlorohydrin--polymer of epichlorohydrin or copolymer of epichlorohydrin and ethylene oxide	8	85	13.6	0.7
Total	3,544		2,022.6	100.0

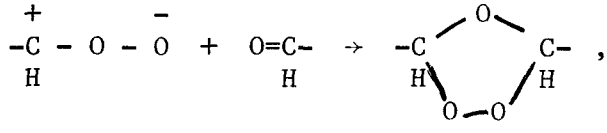
a Derived from Stickney et al.[23]
b Chemical Week, March 18, 1970, p. 58.
c Battelle and industry estimates.

more subject to ozone attack than are saturated elastomers, because ozone attacks aliphatic double bonds. The more highly unsaturated elastomers are listed in Table 13-11. Of these, neoprene is particularly resistant to ozone, owing to the presence of the electronegative chlorine atom in its structure. Elastomers crack under the influence of ozone when stressed, and the crack propogates in a direction normal to the stress direction. The chemical mechanism may be written:[27]



(38)

The zwitterion and aldehydic group are thought to combine,



(39)

to form a highly oxidized structure of much lower extensibility than the unoxidized elastomer. Cracking is the result.

There are two main approaches to avoidance of ozone damage. One is the addition of antiozonants. The more extensively used antiozonants are listed in Table 13-12. These antiozonants have limited solubility in elastomers and tend to "bloom" to the surface. The antiozonant action is not well understood chemically, but probably involves formation of a protective film. Crack initiation occurs at critical stress, which is strongly influenced by the presence of a few percent of the N,N'-dialkyl-p-phenylenediamines. Dithiocarbamates are a second class of antiozonants that

Table 13-11

Highly Unsaturated Elastomers[a]

Polymer	Use	Crack Growth Rate, mm/min[b]	1970 Cost, cents/lb
SBR	General	0.37	23
Natural	General	0.22	25
Polybutadiene	Wear-resistant	ND	25
Neoprene	Oil-resistant	0.01	40
Nitrile	Oil-resistant	ND	50
Polyisoprene	General	ND	24

a Derived from Mueller and Stickney.[6]
b Data from Braden and Gent.[26] ND = no data.

Table 13-12

Elastomer Antiozonants

Antiozonants	Price, cents/lb (1970)
N-N'-Diphenyl-p-phenylenediamine	99
N-N'-Di-(2-octyl)-p-phenylenediamine	90
N-N'-Di-3-(5-methylheptyl)-p-phenylenediamine	90
N-N'-bis-(1,4-Dimethylpentyl)-p-phenylenediamine	86
N-N'-bis-(1-Ethyl-3-methylpentyl)-p-phenylenediamine	90
N-N'-bis-(1-Methylheptyl)-p-phenylenediamine	90

act by slowing crack growth rates. The crack growth rates depend linearly on the concentration of ozone, as shown in Figure 13-2. In addition to antiozonants and special polymeric structures, protection against ozone damage during storage is afforded by the use of waxes, protective coatings, and paper wrapping.

Identity of Oxidizing Species. It is known that atomic oxygen, $O(^3P)$, reacts with double bonds at room temperature to produce a rupture of the carbon-carbon chain. Because elastomers are known to be very stable to ground-state ($^3\Sigma_g^-$) molecular oxygen, the possible oxidants responsible for rubber-cracking in polluted air are atomic oxygen, $O(^1\Delta_g)$, ozone, OH, and HO_2. Relative rates of reaction are products of rate constants and concentrations of oxidizing species.

One elastomer, cis-polybutadiene, has been exposed specifically to $O(^1\Delta_g)$ in the absence of ozone or oxygen atoms.[28] cis-Polybutadiene forms hydroperoxides (-OOH) when exposed to oxygen at 5 torr that has been subjected to an electrodeless discharge (2,450 MHz). Oxygen atoms and ozone were removed by mercuric oxide in these experiments, leaving only $O(^1\Delta_g)$. As we have seen, $O(^1\Delta_g)$ survives for a long time at the low pressures used, nearly half of it surviving 1 s after leaving the discharge zone. No measurements have been made of relative rates of reaction of $O(^1\Delta_g)$, ozone, atomic oxygen, OH, and HO_2 with individual materials, and it is therefore not possible to know the relative importance of attack by these species. The results in Table 13-13 would lead to the speculation that ozone is more important than $O(^1\Delta_g)$ in cis-polybutadiene damage. Because we do not have information on the relative rates of reaction of the five species with elastomers, we can

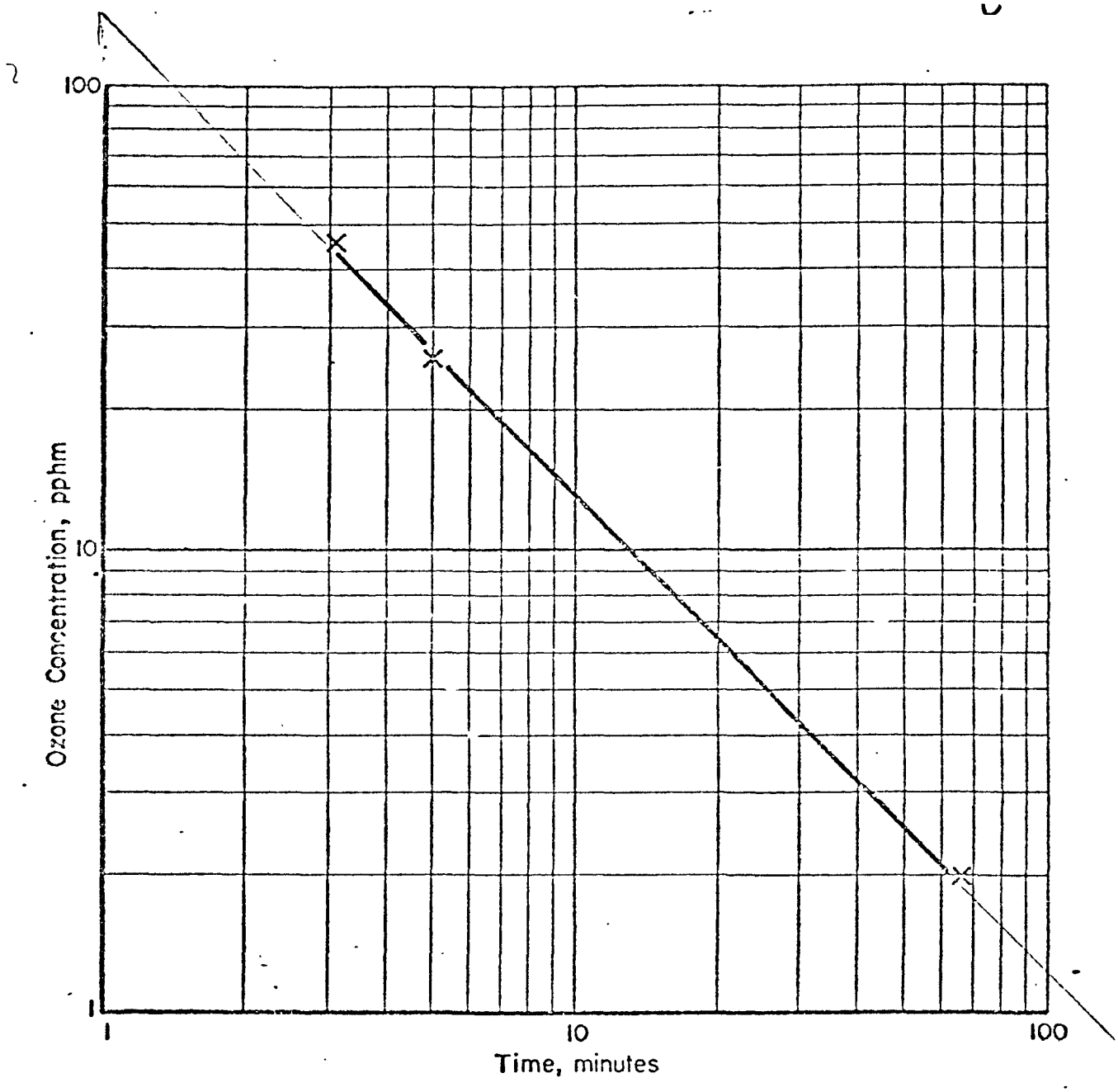


Figure 13-2. Effect of ozone concentration on cracking time. (Reprinted from Mueller and Stickney.[6])

Table 13-13

Rates of Attack of Oxidants on
trans-2-Butene in Simulated Polluted Air

Oxidant	Rate constant (k), molec^{-1} cm^{3} s^{-1} [a]	Concentration, molecules/cm^{3} [b]	Relative Rate
$O_2(^1\Delta_g)$	4.6×10^{-18}	1.4×10^{8}	6.4×10^{-10}
O_3	3.5×10^{-17}	3.8×10^{12}	1.3×10^{-4}
$O(^3P)$	2.3×10^{-11}	2.6×10^{5}	6.0×10^{-6}
OH	2.5×10^{-11}	1.9×10^{6}	4.8×10^{-5}
HO_2	2.4×10^{-11}	1.0×10^{10}	0.24

a Data from Demerjian et al.[18]
b Data from Table 13-4.

assume that the rate constants parallel those of the reactions with trans-butene-2 (see Table 13-3), as was done in estimating the rates of reaction with an olefinic polymer in the laboratory ozone generator. Using the concentrations in Table 13-4 with the rate constants in Table 13-3, we arrive at the attack rates shown in Table 13-13. These results suggest that HO_2 is the most important oxidant in attack on elastomers in ambient air.

Economic Effects. The Battelle study[6] revealed very substantial compounding costs for protection against photochemical oxidants. Table 13-14 summarizes the results of an analysis based on responses to a questionnaire. These costs include the introduction of such ozone-resistant polymers as butyl, neoprene, EPDM, Hypalon, and polysulfide. Fair agreement was obtained between costs obtained from the questionnaire and those from estimates of individual compounding costs as summarized in Table 13-15. In addition to the costs outlined in Table 13-15, other costs are associated with replacement of entire assemblies when a part fails, labor costs for replacement, etc. These costs at the retail level have been estimated by Battelle to total $226 million per year, of which $100 million is attributed to damage of medical goods. In 1969, the cost of antiozonants in automobile tires[2] alone was about $100 million, which represents the cost of avoiding ozone damage to tires. The $226 million estimate does not include labor costs for actual replacement. Because antiozonants tend to discolor, they cannot be used in white sidewall tires, which use, instead, such costly elastomers as Hypalon and EPDM. It is of interest that tires, hoses, and belts do not represent the only important use of rubber in automobiles. Some $50 per car or $500 million per year in other rubber parts represents the value at the manufacturer

Table 13-14

Estimate of Added Manufacturing Costs[a] on National Level[b]

Product	Estimated Value of 1970 Shipments, $ million	Estimated Added Cost per Dollar of Production, mils	Total Added Cost, $ million
Passenger tires	2,352	12	28.2
Truck and bus tires	1,037	12	12.4
Other tires	679	10	6.8
Rubber belts and belting	300	4	1.2
Rubber hose and tubing	480	2	1.0
Sponge and foam-rubber goods[c]	325	--	-----
Rubber floor and wall covering[c]	66	--	-----
Mechanical rubber goods	1,295	2	2.6
Rubber soles and heels[c]	165	--	-----
Drug and medical	100	8	0.8
Footwear	400	1	0.4
Other rubber products	650	1	0.7
Total	7,849		54.1

a Extra costs due to special compounding or formulation to protect against oxidant damage.

b Derived from Mueller and Stickney.[6]

c No protective compounding or formulation used.

Table 13-15

Summary of Added Costs at Manufacturer Level[a]

Type of Cost		Cost, $ million
Special polymers		20.6
Antiozonant		34.1
Wax		5.0
Protective Finishes		
Wrapping		26.0[b]
Compound development		
	Total	85.7

a Derived from Mueller and Stickney.[6]
b Rough estimate.

level. The cost of ozone damage is summarized in Table 13-16. An approximate 25% rise in rubber production was expected between 1969 and 1975. Therefore the cost of ozone damage to elastomers in 1975 should be 1.25 x 596= ~$750 million.

Textile Fibers

It is difficult to find any definitive information that indicates ozone damage as extensive as that suggested in the Midwest Research Institute report.[3] The latter identifies cotton, nylon, and rayon as particularly susceptible to ozone. The oxidation of cellulose fibers by ozone was the subject of a study[29] in 1952 that showed that dry cotton was not seriously degraded by ambient ozone. In more recent studies, these conclusions were confirmed.[30,31] The only information available on fibers other than cotton addressed the effect of ozone on modacrylic, acrylic, Nylon 66 and polyester fabrics.[32] The results indicated minimal effects on these fibers.

However, Jellinek and Chaudhuri[33] exposed Nylon 66 films to nitrogen dioxide, ozone, and ultraviolet radiation. The degree of degradation of polymer, α, was measured by

$$\alpha = \frac{1}{L_t} - \frac{1}{L_o}, \qquad (40)$$

where L_o and L_t are the number-average chain lengths at times o and t, respectively. Degradation proceeds rapidly at 19.2 ppm and stops at around $\alpha = 0.002$ after 1 h. Lower concentrations produce lower ultimate degrees of degradation after longer times (see Figure 13-3). The mechanism of the degradation is believed to be attack at the N-H bond.

Table 13-16

To Elastomers —

Summary of Ozone Damage Cost Per Year (1969)

Type of Cost	Mfr. Level Cost, $ million	Labor Factor	Retail Cost, $ million
Special formulations	85.7	3	257
Early replacements	225.7	1.5	339
Total			596

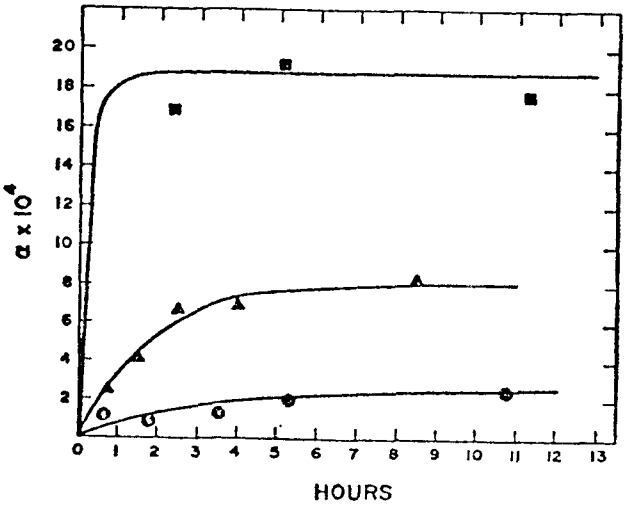
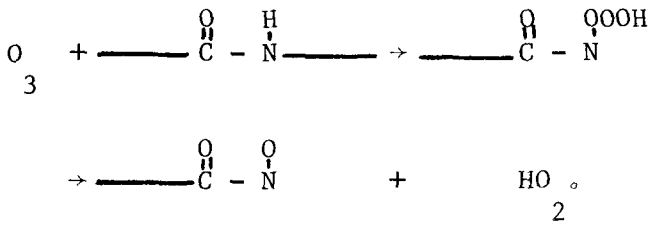
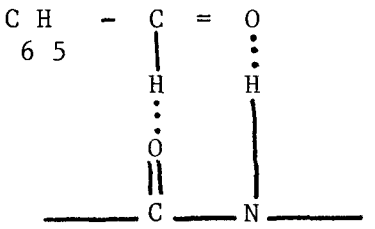


Figure 13-3. Plots of α vs. time for BI films at 35 C in presence of 1 atm of air containing ozone; ■, 19.2 ppm; ▲, 11 ppm; ●, 5.2 ppm. (Reprinted with permission from Jellinek and Chaudhuri.[33])



(41)

Protection of the peptide link is afforded by the addition of hydrogen-bonding compounds, such as benzaldehyde:



The overall evidence of ozone damage to fibers is not compelling.

Dye Fading

O-Fading. The discovery that dyes faded under the influence of ambient ozone was made by accident in 1955.[34] The dye molecule is converted by oxidation to much less deeply colored molecules. The phenomenon became known as "O-fading." Laboratory tests had shown a new dye, Disperse Blue 27, to be resistant to attack ("gas fading") by nitrogen dioxide. Field tests to confirm the laboratory findings were run on acetate draperies containing this dye in a high-nitrogen dioxide area (Pittsburgh) and a low-nitrogen dioxide area (Ames, Iowa). The draperies in both locations were protected from sunlight. The result of 12 months of exposure was opposite to that expected--the Ames draperies had faded more than the Pittsburgh draperies. Ozone was identified as the damaging pollutant, and O-fading was found to occur only when Disperse Blue 27 was used on cellulose

triacetate and polyester, but not with acrylic and nylon. The dyes most susceptible to O-fading were found to be anthraquinone blues, some reds, and some azo reds. Gas-fading inhibitors (such antioxidants as diphenylethylenediamine and tert-butylhydroquinone) are used to retard fading. The American Association of Textile Chemists and Colorists Committee on Colorfastness of Textiles to Atmospheric Contaminants (RA-33) later conducted extensive tests,[35] and these tests eventually led to the discovery of fading problems due to the effect of ozone on permanent-press fabrics and on nylon carpets.[5] Other very extensive tests on dye fading by air pollutants have been carried out under the auspices of the AATCC Committee RA-33, the EPA,[36,37] and others.[38]

Test procedures established by Committee RA-33 include a test ribbon intended to characterize the ozone content of air to which fabrics are exposed. The test ribbon is dyed to the tertiary gray shade with CI Disperse Blue 27. Committee RA-33 has also established a test ribbon for nitrogen dioxide gas fading.

Fading of Permanent-Press Fabrics. Permanent-press garments are blends of polyester and cotton in a ratio of 50:50 or 65:35. The permanent-press formulation includes a catalyst (zinc nitrate or magnesium chloride), a softener (polyethylene), a nonionic emulsifying agent, and a wetting agent. The anthraquinone blues react with the magnesium chloride catalyst to form a chelate that is soluble in the polyethylene softener and in the emulsifying agent, both of which are contained in the "finish." In the curing process (at 320-340 F,or 160-171 C), disperse dyes (e.g., anthraquinone blues) migrate to the "finish"--i.e., migrate preferentially to the folds and creases.

The dye in this substrate, at folds, fades under the influence of ozone. Storage of garments in the summer in warehouses with open windows has resulted in fading in as few as 10 days.[39] Remedial measures include replacing anthraquinone dyes with azo disperse dyes, avoiding the use of magnesium chloride catalyst, and using different surfactants and softeners.

Nylon Carpeting. Consumer complaints of fading nylon carpeting in the warm, humid areas of Texas and Florida gave rise to the term "Gulf Coast fading."[40,41] Laboratory experiments showed the fading to be due to a combination of ozone and high relative humidity (above 65%). It was found that fading was reduced on nylon fibers textured by dry heat, rather than by steam, which produces a moisture-absorbing fiber structure. Such a structure encourages diffusion of ozone throughout the fiber. Measures have been taken to mitigate Gulf Coast fading, and it is no longer considered to be a serious problem.

Economic Effects. Although measures can be and have been introduced to eliminate dye fading due to ambient ozone, these measures are expensive and represent a minimal cost of pollution damage that would otherwise be sustained.

Oppenheimer[42] and Waddell[43] attribute the preliminary economic summary in Table 13-17 to Salvin. These figures are based on increased costs of fade-resistant dyes, inhibitors, research, and quality control. Also included are costs to consumers of decreased product life. Waddell concluded that the "best" estimate of damage to materials for all air pollutants is about $2.2 billion per year.

Table 13-17

Cost of Dye Fading, 1970[a]

Pollutant	Material		Cost, $ Million
NO_x	Acetates		73
	Rayon		22
	Cotton		22
	Spandex		5
		Subtotal	122
Ozone	Acetates		25
	Nylon Carpets		42
	Permanent Press		17
		Subtotal	84

[a] Derived from Waddell.[43]

Other Materials

A number of rather tenuous arguments suggest ozone damage to such materials as recording tape,[44] asphalt,[45] and dried milk.[46] However, studies on such subjects are scattered and generally uncorroborated. Some studies even discuss beneficial effects of ozone, such as reduction of corrosion rates in steel[47] and improvement in adhesion of ink to polyethylene films.

Polyethylene is a major electric insulating material, and the suggestion that ozone may "disastrously" affect its insulating properties[48] bears examination.

Laboratory studies carried out by Priest and his co-workers[49-51] have demonstrated by means of infrared and other techniques that terminal double bonds in polyethylene end groups are attacked by "ozonized" oxygen to form carboxylic acid groups and by rupture of the polymer chain to produce short-chain dicarboxylic acids. A net gain in weight results.

Razumovskii[52] and his colleagues appear not to have been aware of the work of Priest et al. and prefer to interpret their own results as supporting a mechanism that involves attack of ozone on the CH_2-CH_2 unit in polyethylene. Rate constants for the reactions have not been measured, and no assessment of the role of other oxidizing species has been made. However, it is presumed that a high-pressure ozonizer was used and that ozone was the active species.

It is known that atomic oxygen reacts with polyethylene at room temperature[53] to produce a loss in weight and some morphologic changes. The work of Trozolo and Winslow[54] and of Kaplan and Kelleher[28] suggests that $O_2(^1\Delta_g)$ also interacts with polyethylene to form hydroperoxides. Because

polyethylene is known to be very stable to ground-state ($^3\Sigma_g^-$) molecular oxygen, the possible oxidants responsible for polyethylene damage in polluted air are atomic oxygen, O, O_2 ($^1\Delta_g$), and ozone, OH and HO_2. Relative rates of reaction are products of rate constants and concentrations of oxidizing species in the real atmosphere. Thus the relative rates of reaction of these species with polyethylene could be assessed, if we had rate constants for their reactions with polyethylene. Because we do not, we can assume that the rate constants parallel those of the reactions with butene-2 (see Table 13-3). With the concentrations in Table 13-4 and the rate constants in Table 13-3, we arrive at the attack rates shown in Table 13-13.

These results suggest that HO_2 may be the dominant oxidant that attacks polyethylene or other materials in ambient air.

However, despite the known interactions of oxidants with polyethylene and other polyolefins to form intermediate peroxy radicals,[52] there is no evidence that the chemical reactions go far beyond the surface. In fact, polyethylene is specially treated with the products of a corona discharge to improve the surface adhesion of printing ink.[55] Winslow[56] believes that the effects of atmospheric ozone on polyethylene insulation and other polyethylene products are negligible, compared with the embrittlement of polyethylene by a combination of oxygen and solar ultraviolet radiation. The mechanisms by which this embrittlement occurs probably involve sensitization to oxidation by absorption of ultraviolet radiation by residual hydroperoxy and carbonyl groups in the polymer and by surface

deposits of aromatic sensitizers from polluted air. Deterioration of the electric insulating properties of polyethylene[48] by oxidation in some environments cannot be attributed to ozone. Damage to polyethylene by ozone suggested by the figures in Table 13-5 is undoubtedly overestimated.

In view of the relative stability of polyethylene toward ozone, it is surprising that the perfluorinated analogue (Teflon) reacts with ozone to produce perfluoroformaldehyde and carbon dioxide.[57] Because Teflon is widely used to contain ozone-air mixtures, researchers are cautioned to be aware of this reaction.

SUMMARY

In the context of this review, the term "photochemical oxidants" is considered to be synonymous with "ozone." In test chambers that have external ozone generators and that operate at or near atmospheric pressure, ozone is the only likely oxidizing species. In ambient air, however, ground-state atomic oxygen, hydroxyl radicals, and especially hydroperoxy radicals can compete with ozone in attacking materials, such as rubber, that contain olefinic bonds. The most economically important materials with respect to ozone damage are paint, elastomers (rubber), and textile fiber-dye systems. Damage to polyethylene by ozone is considered to be negligible. The ozone-specific damage in 1970 to materials has been assessed in economic terms and is approximately as follows: paint, $540 million; elastomers, $569 million; and textile fibers and dyes, $84 million. Total material damage attributable to ozone is, therefore, $1.22 billion. This is to be compared with Waddell's estimate of total air pollutant material damage of $2.2 billion. It is clear that oxidants are very important molecules in pollutant damage to materials.

RESEARCH RECOMMENDATIONS

- Laboratory studies of effects of photochemical oxidants other than ozone--e.g., PAN, peroxybenzoylnitrate, atomic oxygen, excited molecular oxygen, $O_2(^1\Delta_g)$, and hydroperoxy and hydroxyl radicals--on specific materials should be conducted.
- Concentrations of oxidants other than ozone should be measured in real atmospheres.
- Mechanisms of attack of oxidants on materials should be investigated, so that distinctions can be made between effects on a given material due to various pollutants.
- An integrated study should be conducted in which relative effects of all major air pollutants on materials are assessed.

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CHAPTER 14.

GENERAL SUMMARY AND CONCLUSIONS

The general summary and conclusions that appear below is a collage taken from the individual chapters.

CHEMICAL ORIGIN

The major primary pollutants of importance to oxidant formation are nitric oxide, hydrocarbons, aldehydes, and carbon monoxide. A few free radicals are formed by photolysis of aldehydes and nitrous acid by sunlight or by the reaction of traces of ozone with reactive hydrocarbons. These free radicals initiate chain reactions involving hydroperoxy and alkylperoxy radicals. During these chain reactions, the nitric oxide is converted to nitrogen dioxide, and the hydrocarbons and aldehydes are degrated. The photolysis of nitrogen dioxide by sunlight forms a free oxygen atom, which combines with an oxygen molecule to form ozone. Because of the $NO-NO_2-O_3$ cycle (Eqs. 1-3/) the ozone concentration is determined primarily by the ratio $[NO_2]$: [NO] and so does not become large until most of the nitric oxide has been converted to nitrogen dioxide. The total amount of oxidant formed depends, in a nonlinear fashion, on the amount of hydrocarbons available to continue pumping the nitric oxide to nitrogen dioxide. Aldehydes and even carbon monoxide can also serve this pumping function. When some of the peroxy radicals recombine or react with the nitrogen oxides, many secondary products, such as hydrogen peroxide and PAN, are formed.

Recent chemical modeling studies have been reasonably successful in reproducing the concentration -- time histories of smog-chamber experiments. An examination of these models shows a need for much more detailed chemical knowledge. Modeling studies also point out the necessity of carefully defining the initial conditions of smog-chamber experiments. Some observations that have been made with these models are:

- Even if hydrocarbons are completely removed from the air, aldehydes and NO_x can generate high concentrations of photochemical oxidants.
- If both hydrocarbons and aldehydes are eliminated, carbon monoxide and NO_x alone can generate significant concentrations of ozone.
- The concentration of ozone generated photochemically goes through a maximum as the NO_x concentration is increased.
- The steady-state concentration of free radicals in smog is approximately 0.3 ppb and is rather insensitive to primary pollutant concentration.

The concentrations, average lifetimes, and rates of attack of the reactive intermediates can be calculated with chemical models.

The effects of free radicals on biologic surfaces cannot be ignored.

The development of lasers has opened up several new techniques for monitoring pollutants in the atmosphere. Sensitivities down to the parts-per-billion range are claimed, and continuous monitoring is possible. The photoionization mass spectrometer has been developed as a sensitive detector for free radicals in the gas phase. A high-resolution mass spectrometer coupled to a computer is capable of detecting up to 300 compounds in air, both in particulate form and in the gas phase.

AEROSOLS

Review of the literature provides ample evidence that aerosol formation is an important part of the atmospheric chemistry linked with photochemical oxidant production. The important chemical constituents of concern include sulfate, nitrate, and secondary organic material.

Secondary organic aerosols, formed by gas-phase reaction between nitrogen oxide, ozone, and hydrocarbons constitute an important fraction of urban photochemical smog. Data obtained at high ozone concentrations

(0.67 ppm) can be taken as an upper limit of the contribution of secondary organic aerosols to the organic aerosol fraction and total suspended particulate material (95% and 65%, respectively). Most of the identified ambient secondary organic aerosols are difunctional compounds that bear carboxylic, nitrate, aldehyde, and alcohol groups. The same compounds have been identified in smog chambers from C_{5+} cyclic olefins and diolefins, with gas-to-aerosol conversion factors exceeding by more than an order of magnitude those measured for the ambient average conversion of all reactive hydrocarbons. The formation of such species in the gas phase in excess of their saturation concentration followed by condensation on preexisting particles and further growth in the light-scattering range is the predominant physical mechanism that controls the gas-to-aerosol conversion process.

Because of their very low vapor pressures, difunctional compounds are readily converted to the aerosol phase, whereas more volatile monofunctional compounds require much higher precursor and ozone concentrations to reach their saturation concentration. This explains why most of the compounds formed from alkenes remain in the gas phase, whereas C_{5+} cyclic olefins and diolefins are efficient aerosol precursors. However, there is no known source of the latter class, so cyclic olefins, identified in both gasolines and auto exhaust, can be regarded as the most important source of secondary organic aerosols. The role of aromatics as aerosol precursors is essentially unknown. Because of their accumulation in the submicrometer range, all secondary organics are potentially dangerous. However, there is almost no information on health effects associated with the presence of such compounds in the atmosphere.

Because the conclusions presented in this chapter rely heavily on a few recent studies, it is extremely difficult to relate the urban con-

centrations of secondary aerosols to the concentration of their gas-phase precursors. Simple relations of the type d(secondary aerosol)/dt = α (precursor) (ozone) have been derived from smog-chamber data for organic aerosol formation in mixtures of cyclic olefins and NO_x and sulfate aerosol formation in mixtures of NO_x, sulfur dioxide, and C_{5-} alkenes. Such kinetic data are consistent with the organic (a few micrograms per cubic meter per hour) and sulfate (up to 13%/h) aerosol formation rates observed in photochemically polluted urban areas. More complex kinetic relations reflect certainly all the possible variations between these extreme and rather simple systems. Although control of ozone, through control of NO_x and <u>total</u> hydrocarbon emission, would obviously have a roughly proportional effect on the formation of organic aerosols, present data suggest the identification and control of a few <u>specific</u> hydrocarbon precursors as an alternative approach. The contribution of photochemical reactions involving hydrocarbons to inorganic nitrate and sulfate aerosol formation remains to be determined. More data on the identification of hydrocarbon precursors and on the kinetics of formation, physical parameters, and health effects of their products would ultimately permit quantifying the complex relations between secondary aerosols and ozone concentrations in urban atmospheres.

ATMOSPHERIC CONCENTRATIONS OF PHOTOCHEMICAL OXIDANTS

In comparison with previously available material on atmospheric concentrations of photochemicaloxidants, we now have a far richer data base and a deeper understanding of how to interpret the reported concentrations. The recent information on hydrogen peroxide and the broader geographic coverage of measurements abroad are examples of new data that have come to light.

Subtleties in future standard-setting must consider receptor damage in terms of exposure location and time and receptor distributions and response functions. The formula for damage function points up the need for improved knowledge of spatial and temporal distribution. The use of second-to-worst hourly readings for an ambient air quality standard must give way to a specification stated in terms of a statistically defensible higher-frequency event. This will reduce substantially the uncertainty inherent in confining one's attention to the "worst case."

Long-term trends in oxidant concentration cannot be identified with nearly the degree of certainty that we might like. The data suggest a decrease in oxidant concentrations in central-city areas and an increase in downwind areas. Measurements of nonurban oxidant are exhibiting a higher frequency of violations of the ambient standard than was once believed to occur. Figure 4-43 shows typical ranges of concentrations in various regions in and out of urban complexes.

Probably the most critical question today regarding atmospheric concentrations of ozone and other photochemical oxidants is, "What fraction of the observed values in each locale is susceptible to control by anthropogenic emission reduction?" As brought out in this chapter, there is one school of thought embracing the idea that nature frequently presents us with concentrations that exceed the U.S. national ambient air quality standards. The other point of view is that global background ozone concentrations do not exceed 0.05 - 0.06 ppm at the surface and that higher concentrations than this have anthropogenic sources.

The data presented in the literature reviewed above support the second point of view. The observation of ozone concentrations exceeding the ambient standard in nonurban areas does not demonstrate that this is of natural

origin. However, the measurements in remote areas of the northern hemisphere, compared with those in the continental United States, do support the thesis that anthropogenic sources are involved in cases where the standard is exceeded.

This is a very broad conclusion, and additional measurements must be made. Some of this effort (which is goingon) should address the problem of other pollutants and condensation nuclei that accompany the nonurban oxidant. Interpretation of these measurements will increase the specificity of separating anthropogenic sources from natural background sources. Theoretical assessments of the existing observations will shed light on the relative roles played by stratospheric injection, plant emission, background methane, and dry deposition on surfaces in the natural portion of the tropospheric ozone cycle.

Geographically, our best measurements have focused on the Los Angeles, California, region because of the severity of the problem there. The Regional Air Pollution Study and its extensions will, it is hoped, supply an additional rich data base for the St. Louis, Missouri, region. Airborne-pollutant measurements aimed at specific experimental objectives are needed in the central and eastern areas of the United States to broaden the foundations of a national control strategy. Existing ground-based continuous monitoring networks will not provide an adequate basis for the regional control of oxidant. Ad hoc, one-shot aircraft measurements have led mainly to speculation that can establish incorrect attitudes on the origin and fate of nonurban ozone. In the vigor of environmental control efforts, incomplete data sets have stimulated hasty targeting on specific sources (for example, rural vehicular emission, power stations, trees, and frontal passages).

Another subject of recent interest has been the question of indoor-outdoor oxidant concentrations. Available measurements and models suggest that indoor exposures may be substantially reduced by appropriate choices of ventilation systems, air filters, and interior surface materials. The cost-benefit relationships coming from these studies may well have a great impact on future decisions based on atmospheric concentrations of oxidant pollutants.

Much care must be exercised in comparing atmospheric concentrations between one place and another because of differences in primary calibration techniques or in instrumentation. Chapter 6 summarizes these problems in detail.

MODELS FOR PREDICTING AIR QUALITY

The literature contains reviews of air quality modeling that stress special purposes. Some concentrate on meteorologic aspects, and others combine this with air chemistry. Proceedings of several conferences are another information resource. Recent surveys have been addressed specifically to photochemical modeling problems. It may be concluded that, although they are relatively complex, the photochemical-diffusion models perform as well as, if not better than, available inert-species models.

A variety of goals and objectives may be met with air quality modeling. They are summarized as:

- Scientific understanding of atmospheric phenomenology.
- Rational application of the regulatory process.
- Land-use planning within environmental constraints.
- Real-time control of episodes.

The fundamental elements of deterministic models involve a combination of chemical and meteorologic input, preprocessing with data transmission, logic that describes atmospheric processes, and concentration-field output tables or displays. In addition to deterministic models, there are statistical schemes that relate precursors (or emission) to photochemical oxidant concentrations. Models may be classified according to time and space scales, depending on the purposes for which they are designed.

Specific model applications to the oxidant problem include both the simple rollback (with modifications) and the photochemical-diffusion techniques. Very little modeling of intermediate complexity seems to have been attempted for the oxidant system.

Model performance is now receiving critical attention because of the need for cost-effective control measures. Standard statistical performance descriptors can sometimes mislead a prospective user; therefore, more specialized tests are being devised. Various model types are being compared for a specified set of initial and boundary conditions. It is apparent from these studies that added fidelity is purchased at the expense of added complexity of a logical structure that must represent the controlling phenomenology.

MEASUREMENT METHODS

With the exception of calibration, the measurement problems which were apparent in 1970, at the time of publication of the first air quality criteria document on photochemical oxidants, have essentially been solved for ozone. This remarkable achievement is the result of unstinting efforts by individuals working at EPA's National Environmental Research Center, North Carolina; the National Bureau of Standards; private research contractors sponsored

primarily by EPA; private instrument manufacturers; the Jet Propulsion Laboratory of the California Institute of Technology; the Air and Industrial Hygiene Laboratory, California Department of Health; the Air Pollution Research Center of the University of California at Riverside, and the California Air Resources Board (CARB).

Due to the focus on the problem brought about by the CARB , a significant advance in the accurate calibration of instruments for monitoring ozone in ambient air was achieved during 1975. As a result, this agency adopted the measurement of ozone in the ultraviolet region at 254 nm as a primary calibration reference standard. They have also adopted statewide, as a transfer standard for calibrating ozone and oxidant monitoring instruments at air monitoring stations, a commercially available instrument (coupled with the precise controlled generation of ozone in air), which measures the differential absorption of ultraviolet radiation.

It is important to separate conceptually, and in practice, the calibration process from the monitoring process. Photochemical oxidants consisting primarily of ozone were first continuously measured in Southern California by measuring the color change of potassium iodide solutions brought into contact with the ambient air. This measurement continues to yield valid photochemical oxidant data in California. However, it has yielded questionable data at ambient air monitoring sites elsewhere in the United States. For this reason at the end of 1971, EPA officially adopted a continuous monitoring process that measures the chemiluminescence produced when ozone in air is brought into contact with the gas ethylene. This reference procedure when calibrated with the primary reference procedure using ultraviolet absorption is widely accepted.

Instruments based on differential ultraviolet absorption still need to be evaluated, and possibly modified, prior to their acceptance for monitoring ozone in polluted atmospheres on a nationwide scale. The California Air Resources Board and other air pollution control agencies are currently conducting multi-year programs for evaluating ultraviolet absorption side-by-side with chemiluminescent and potassium iodide based instruments, to determine their applicability and needed modification, as well as to assure continuity in the data base while the older monitoring instruments are being replaced.

Thus despite the remarkable progress in the monitoring for ozone, nitrogen oxides and non-methane hydrocarbons, which has strengthened the implementation and evaluation of control programs, substantial research and development is still required to help resolve the uncertainties in our knowledge which are inhibiting the actual achievement of desired air quality standards.

RESPIRATORY TRANSPORT AND ABSORPTION

This chapter has discussed the general approach required to model the transport and absorption of ozone and other pollutant gases in the respiratory tract. For unreactive or very weakly reactive gases, there are a few models that are qualitative descriptions for assessing total dosage and dosage in major regions. However, there is no adequate model for gases like ozone, which are strongly reactive within the mucous and tissue layers.

TOXICOLOGY

The acute lethal action of ozone is due to its capacity to produce pulmonary edema. The LC_{50} for rats and mice exposed for a single 4-h period is approximately 6 ppm, and cats, rabbits, guinea pigs, and dogs (in that order) are decreasingly susceptible to the lethal action. Numerous reviews have considered the toxicity of ozone at lethal and lower concentrations.

In the foregoing discussion and in Table 14-1, attention has focused on studies in laboratory animals that have been exposed to ozone concentrations of about 1 ppm or less, because results of studies conducted with such concentrations are thought to be more directly relevant to ambient oxidant air pollution, which is the source of exposure for large human populations. In Table 14-1, no attempt has been made to list all studies; more comprehensive summaries can be found in some of the monographs cited. Instead, the table cites studies thought to be most useful for evaluating the health implications of exposure to low concentrations of ozone, and the concentrations listed are the lowest at which the described effects have been observed. Unfortunately, many studies have not included a sufficient range of experimental concentrations to permit construction of reliable dose-response curves or to determine what, if any, would be a "no-observed-effect" concentration for the experimental conditions used. Although it is understandable that research scientists find little stimulation in conducting exposure experiments at concentrations that fail to produce changes in the biologic system in which they are interested, the conduct (and reporting) of such experiments is extremely important for a pragmatic evaluation of the implications of positive findings for human health.

The table illustrates the wide variety of biologic effects produced in laboratory animals exposed to relatively low concentrations of ozone. Obviously, some effects have more serious health implications than others.

Table 14-1

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal
0.03	3 h	Increased mortality from pulmonary infection with Streptococcus	Mouse
0.1	7 h/day, 5 days/wk for 3 wk	Increased incidence of neonatal mortality in litters of exposed parents (related effects and reduced fertility noted in synthetic oxidant smog)	Mouse
	7 days	Increased succinate-dependent lung mitochondrial oxygen consumption in rats on diet relatively low in vitamin E; no significant effect in vitamin E-replete rats at this concentration of ozone	Rat
0.2	3 h	Degenerative changes in Type I alveolar cells; later replaced by Type II cells	Rat
	Continuous for 28-32 days	16% increase in lung volumes; overdistention at high lung volumes, suggesting some change in elasticity; no change in respiratory frequency, tail length, or external appearance	Young rat
	6 h or continuous for 7 days	Decreased voluntary runway activity during exposure; no-effect concentration not reported; reduced gross motor activity	Mouse
	7 h/day, 5 days/wk for 3 wk	Increased incidence of blepharophimosis and jaw abnormalities in neonates	Mouse
	7 days	Increased succinate-dependent lung mitochondrial oxygen consumption	Rat

Table 14-1 (cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal
0.2 (Cont.)	Continuous for 8 days	Dose-related increased activity of lung glutathione peroxidase and glutathione reductase (glucose-6-phosphate dehydrogenase activity significantly increased at 0.5 ppm)	Rat
	5 h	Lymphocyte chromosomal breaks	Hamster
0.2-0.25	0.5-2 h	Increased red-cell spherocytosis after *in vitro* radiation	Mouse, rat, rabbit, man
0.25	4-6 h	Morphologic changes in medium-sized airways	Cat
0.25-0.5	6 h	Threshold for lung edema formation with ^{131}I albumin test	Rat
	3 h	Decreased lysozyme, acid phosphatase, and β-glucuronidase activity in alveolar macrophages (appears to be linearly related to dose up to 1 ppm)	Rabbit
0.26-0.5	4.6 h	Increased lung flow resistance in 2 animals at 0.26 ppm; effect in all at 0.5 ppm	Cat
0.34	2 h	30% increase in frequency of breathing; 20% decrease in tidal volume	Guinea pig
0.37-0.5	2 h	Decreased red-cell acetylcholinesterase and increased osmotic fragility (no effect at 0.25 ppm)	Man
0.4	6 h/day, 5 days/wk for 10 months	Increased serum trypsin protein esterase	Rabbit
0.4-0.7	4 h	Conjugated diene bonds, suggesting lung lipid peroxidation	Mouse

Table 14-1 (cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal
0.5	6-10 h	Minor chromosomal abnormalities	Man
	3 h	Inhibition of intracellular hydrolytic enzymes of alveolar macrophages; increased fraction of polymorphonuclear leukocytes	Rabbit
	165 min	Alterations in blood, including red-cell membrane and enzyme changes and increased serum vitamin E and lipid peroxides	Man
	6 h	Decreased lung DNA synthesis	Mouse
0.5-1	1 h	Decreased electric response of specific areas of brain with evoked-response technique	Rat
0.54-0.88	Continuous for up to 3 wk	Morphologic changes in distal and respiratory bronchioles, alveolar ducts, and associated alveoli	Young rat
0.6	Not applic.	Avoidance of cage ventilated with ozone	Mouse
	93 days	No change in behavior or chronaxial muscle ratios	Rat
0.68	4 h	Decreased rate of bacterial killing in lungs *in vivo*	Mouse
	2 h	No significant increase in respiratory flow resistance	Guinea pig
0.7-0.8	Continuous for 7 days	Increased acid phosphatase in specific lung areas determined histochemically	Rat

Table 14-1 (cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal
0.7-0.8 (Cont.)	Continuous for 5-7 days	Increased activity of lysosomal hydrolases in whole-lung homogenates	Rat
0.75	3 h	Decreased benzopyrene hydroxylase in lung and tracheobronchial mucosa	Hamster
	4-8 h	Histologic changes in parathyroid glands	Rat
0.8	Continuous for 7 days	Histochemically determined alteration in several lung enzyme activities	Rat
	Continuous for 8 days	Increased activity of lung and plasma lysozyme (no effect at 0.2 and 0.5 ppm)	Rat
	7 days	Increased activity of lung pentose shunt and glycolytic enzymes; decreased lactic dehydrogenase	Rat
0.84	4 h/day, 5 days/wk for 2 wk	Increased susceptibility to respiratory infection with Klebsiella pneumoniae	Mouse, hamster
0.85	4 h	Heinz bodies in circulating red cells; further exposure led to decrease in Heinz body formation	Mouse
0.9	Continuous	Lungs 38% heavier than those of normals; 50% dead in 3 wk	Young rat
1	1 h	Chemical changes in ground substance and lung protein	Rabbit
	4 h	Engorged blood vessels and excess leukocytes in lung capillaries	Mouse

Table 14-1(cont.)

Summary of Effects of Exposure of Laboratory Animals to Ozone at Low Concentrations[a]

Ozone Concentration, ppm	Duration of Exposure	Observed Effects	Animal
1 (Cont.)	90 min	Decreased lung cytochrome P-450	Rabbit
	1 h	Formation of carbonyl compounds and alterations of hyaluronic acid in lung	Rabbit
	8-24 h/day for 18 months	Alteration in catechol-O-methyltransferase and monoamine oxidase of brain tissue	Dog
	3 h/day for 2-3 successive days	Prolongation of phenobarbital sleeping time (no effect after 1 or 4-7 days)	Mouse
	Continuous for up to 18 months	Bronchitis; bronchiolitis; emphysematous and fibrotic changes; acceleration of lung-tumor development	Mouse
	Continuous for 1 wk	Decreased voluntary running activity	Rat
1-3	Continuous for 18 months	Thickening of bronchioles and respiratory bronchioles; barely noticeable at 1 ppm; at 3 ppm, formation of peribronchiolar collars with resulting narrowing of small airways	Dog
1.08	2 h	47% increase in respiratory flow resistance	Guinea pig
1.3	3 h	Increased susceptibility to Klebsiella pneumoniae	Mouse, hamster

[a] Concentrations listed are lowest for which observed effects have been reported.

CONTROLLED STUDIES ON HUMANS

Convincing new information on the health effects of oxidant exposure has emerged from controlled studies on humans, from which tentative dose-response curves have been constructed. The new data show statistically significant reduced pulmonary function in healthy smokers and nonsmokers at ozone concentrations at and above 0.37 ppm for 2-h exposures. Other gases and aerosols found in an urban atmosphere were not present in these experiments. Some studies suggest that mixtures of sulfur dioxide and ozone at a concentration of 0.37 ppm are more active physiologically than would be expected from the behavior of the gases acting separately.

Wide variation in response among different individuals is a general finding in studies of oxidants, as well as other pollutants.

Undesirable health effects of oxidant air pollution exposure are increased by exercise, and many people apparently limit strenuous exercise voluntarily when oxidant pollution is high.

Safety, ethical, and legal considerations require that the utmost care be exercised in human experimentation. The risk inherent in this work can be minimized by taking reasonable precautions while ensuring the satisfactory performance of the study.

PLANTS AND MICROORGANISMS

Oxidant injury to vegetation was first identified in 1944 in the Los Angeles basin. Our understanding of oxidant effects and of the widespread nature of their occurrence has increased steadily since then. Although the major phytotoxic components of the oxidant (photochemical) complex are ozone and peroxyacetylnitrate (PAN), indirect data support the contention that other phytotoxicants are present in the photochemical complex. Ozone is considered the most important phytotoxic component and was first identified as the specific cause of weather fleck on tobacco and stipple on grape. PAN is associated with the undersurface glazing and bronzing associated with many of the vegetable crops.

Plant response to oxidants (including ozone and PAN) is often divided into visible and subtle effects. Visible effects are identifiable pigmented, chlorotic, and necrotic foliar patterns resulting from major physiologic disturbances. Subtle effects produce no visible injury, but include metabolic disturbances and may be measured on the basis of growth and long-term biochemical changes. These effects may influence plant populations and communities and could have an adverse influence on ecosystems. Visible injury may be acute or chronic. Acute injury breaks down the cell membrane and causes cell death, with leaf necrotic patterns that may be characteristic for a given oxidant, but can be confused with other stress factors. Classic injury from ozone is the upper-surface fleck on tobacco and the stipple of grape. Many plants show an upper-surface bleach with no lower-surface

injury. Bifacial necrotic spotting is common and may appear flecklike. Classic injury from PAN appears as a glaze followed by bronzing of the lower leaf surface in many plants. Complete collapse of leaf tissue can occur, if concentrations are high. Chronic injury is associated with disruption of normal cellular activity followed by chlorosis or other color or pigment changes that may lead to cell death. Chronic injury patterns are generally not characteristic and may be confused with symptoms caused by biotic diseases, insects, nutritional disorders, or other environmental stresses. Early leaf senescence and abscission may result from chronic exposure.

Leaf stomata are the principal entry sites for ozone and PAN. Stomata, when closed by any of a number of factors, will protect plants. Ozone and PAN may interfere with various oxidative reactions in plant cells. Membrane sulfhydryl groups and unsaturated lipid components may be primary targets of oxidants. Physiologic leaf age is an important consideration in the response of the leaf to oxidants. Young leaf tissue is more sensitive to PAN, whereas newly expanding and maturing tissue is most sensitive to ozone. Light is required before plant tissue will respond to PAN, but not to ozone. Oxidants affect such physiologic processes as photosynthesis, respiration, transpiration, stomatal opening, metabolic pools, biochemical pathways, and enzyme systems. The acute response of plants to ozone and PAN may result from a saturation of sensitive cell sites and a disruption of normal cellular repair mechanisms. Chronic injury probably results from secondary reactions involving membrane injury.

There is evidence that ozone is a radiomimetic gas. Ozone affects pollen germination in some species and thus may directly affect yield. A study with Arabidopsis thaliana suggested no mutagenic effects from ozone on this plant over five generations.

Ambient-oxidant studies in filtered versus nonfiltered field chambers have reported up to 50% reduction in citrus yield (orange and lemon), a 10 - 15% reduction in grape yield in first year and 50 - 60% reductions over the following 2 years, and a 5 - 29% reduction in yield of cotton lint and seed in California. Losses of 50% in some sensitive potato, tobacco, and soybean cultivars have been reported from the eastern United States. It is apparent that ambient oxidants do reduce yields of many sensitive plant cultivars. Growth reductions associated with acute exposure to ozone are often associated with injury; sometimes the correlations are high. Even multiple exposures and sometimes chronic exposures have shown fair to good correlation between injury and growth (biomass) reductions. The greater reductions in root growth than in top growth reported in several species are related to solute transport and may be fairly common under some conditions. Ozone affects nodule number, but not nodule efficiency in clover, soybean, and pinto bean. This causes a reduction in nitrogen fixation associated with legumes and, if widespread, could have a major impact on plant communities and affect fertilizer needs. The effect on nodulation is related to carbohydrate supply. Yield reductions with little injury after chronic exposure are known for several crops. Severe injury in tomato was required before a yield reduction was found. Chronic exposures to ozone at 0.05-0.15 ppm for 4-6 h/day will produce yield reductions in soybean and corn grown under field conditions. The threshold appears to be 0.05-0.10 ppm for some sensitive cultivars and is well within values monitored in the eastern United States. Growth or flowering effects, at chronic exposures to ozone at 0.05-0.15 ppm for 2-24 h/day are reported for carnation, geranium, radish, and pinto bean grown in greenhouse chambers.

Plant sensitivity to ozone, PAN, and other oxidants is conditioned by many factors. Genetic diversity between species and between cultivars within a species is well documented. The mechanism of genetic resistance is known for only one onion cultivar and is related to the effect of ozone on stomatal closure. Variants within a natural species are well known for several pine species, including white, loblolly, and ponderosa. Plant sensitivity to oxidants can be changed by both climatic and edaphic factors. A change in environmental conditions will initiate a change in sensitivity at once, but it will be 3-5 days before the response of the plant is completely changed. Plants generally are more sensitive when grown under short photoperiods, medium light conditions, medium temperature, high humidity, and high soil moisture. Injury to PAN may increase with increasing light intensity. Conditions during exposure and growth affect the response of plants to oxidants in similar ways. However, plants exposed to ozone are more sensitive to increasing light intensity and, in some cases, to decreasing temperature during the exposure period. In general, growth factors that tend to cause a physiologic hardening of plant tissue make the plants more tolerant to ozone. At the time of exposure, factors that increase water stress tend to make plants more tolerant to ozone. Soil moisture is probably the most important environmental factor that affects response during the normal growing season.

Plants respond in different ways to pollutant mixtures; less than additive, additive, and greater than additive effects have been reported. Mixtures of ozone with sulfur dioxide and of nitrogen dioxide with sulfur dioxide can cause oxidant-like symptoms in some sensitive plants. Mixtures can cause effects below the threshold for either gas, although there is some

disagreement on this in regard to ozone. Ratios of mixtures, intermittent exposures, sequential exposures to pollutants, and predisposition by one pollutant to the effects of a second pollutant may all be important in nature, but little research has been done.

The response of some plants to oxidants is conditioned by the presence or absence of biotic pathogens. Depending on the plant and the pathogen, oxidants may cause more or less injury to a given species. Pathogens may protect their host or make it more sensitive. The pathogens themselves may be injured or may be protected by the host plant. This subject is just starting to be understood.

Oxidant injury to ponderosa pine predisposes the trees to later invasion by pine bark beetles. Ozone and ozone-sulfur dioxide mixtures may decrease the population of soybean nematodes. Both greater and smaller effects have been noted when herbicides have been used in the presence of high oxidant concentrations.

The two most critical factors in terms of air quality standards are duration of exposure and concentration. These two factors determine the exposure dose for a plant. In determining the response of vegetation, concentration is more important than time. A given dose presented to a plant in a short period has a greater effect than the same dose applied over a longer period. This suggests a threshold effect for plant populations and is probably related to the repair mechanisms inherent in biologic systems. Sufficient information is not available from long-term chronic studies, but a threshold between 0.05 and 0.10 ppm is probable for ozone (oxidant). For acute effects, an overall threshold concentration with respect to time can be determined from Figures 11-6 and Table 11-24a. For pinto bean (Table 11-22), this threshold

for injury is about 0.03 ppm for 8 h and about 0.10 ppm for 1 h. This suggests that oxidant standards may be needed for periods up to 8 h.

Vegetation can act as a major sink for oxidants over time, but has a relatively minor effect on oxidant concentrations during episodes of high air pollution, is more effective at some seasons or under some cultural and management practices than others, and should not be considered an important contributor to short-term reductions in oxidant or ozone concentrations.

Plant protection from air pollution stress has involved three types of programs. Several researchers are including pollutant stress in standard breeding programs with the aim of developing resistant cultivars. Our present concepts of pollution effects suggest that the gene pool of all species is large enough to permit the development of more tolerant cultivars. Natural selection will slowly do this for native vegetation. If pollution concentrations go no higher, this should be an effective protection device. Interim measures involve the use of chemical sprays. Such sprays are not yet economically feasible, but several do give adequate protection against oxidants. Fungicides, such as Benomyl, may serve a dual function. Cultural and land use practices may also play important roles, especially on a short-term basis.

Little research on the effects of oxidants on nonvascular green plants and microorganisms has been reported. Lichens and mosses are responsive to acid gases, but there is no definite evidence that they respond to oxidants. Ferns may be especially sensitive, but their injury response is much different from that of higher plants. Growth and sporulation of fungi on surfaces are usually, but not always, affected. Ozone does not penetrate the leaf tissue or the colony and thus does not cause death of colonies. Ozone from

0.1 to several milligrams per liter of solution is required to kill many microorganisms in liquid media. Most work with microorganisms has been done to study the effectiveness of ozone as a biocide in the storage of vegetation or treatment of water or sewage supplies.

Plants have been used as biologic indicators of oxidant pollutants for many years. Attempts have been made to use plants as monitors, but too many unknown variables are involved. Plants may be capable of monitoring the total biologic potential for adverse effects, but no research has been developed along these lines.

Losses based on farm prices are not appropriate at the consumer level and are likely to be conservative. Because of percentage markups and fixed wholesale and retail marketing costs, the cost to the consumer from agricultural losses to oxidant pollutants could be as much as $600 million per year.

ECOSYSTEMS

The transport of injurious concentrations of ozone and other oxidants to rural areas downwind from urban centers at numerous locations in the United States appears to be on the increase. Blumenthal *et al.* conservatively estimate that the urban plume from the Los Angeles area "could cause ozone concentrations to exceed the Federal standard of 0.08 ppm at locations as far as 260 km." Other areas where significant rural concentrations of oxidant have been observed are Salt Lake City, Denver, and the Blue Ridge Mountains.

In general, the permanent vegetation constituting natural ecosystems receives much greater chronic exposure, and the short-lived higher-value vegetation constituting the agroecosystem of the Los Angeles coastal plain

can be subject to injurious doses, but in intermittent short-term fumigations. Each situation has measurable economic and aesthetic effects, but on different time-scales. The simple agroecosystem has little resilience to pollutant stress; losses are immediate and sometimes catastrophic. The complex natural ecosystem is initially more resistant to pollutant stress, but the longer chronic exposures cause disruption of both structure and function in the system that may be irreversible.

Simulation models of ecosystem subsystems are developing rapidly. They deal with flows of energy, biomass, mineral nutrients, water, numbers of species, population densities, and area occupied per biotic unit. Interactions between ecosystem components must be understood before prediction can be attempted. Simulation models offer a bright opportunity for determining the long-term effects on natural ecosystems and agroecosystems. New knowledge of biologic effects should suggest the importance of prevention and some means for ameliorating damage.

Oxidant injury to the mixed-conifer stands of the San Bernardino Mountains beginning in the early 1940's is well advanced. A similar problem is developing in the forests of the southern Sierra Nevada. Both places show both direct and indirect effects on all subsystems of the forest ecosystem -- producers, consumers, and decomposers. For example:

- Ozone injury limits biomass production by the primary producers and their capacity to reproduce.
- The decrease of biomass or energy flow to consumer and decomposer in the ecosystem affects the populations of these organisms.

- Essential recycling processes, such as recycling of nutrients, may be interrupted, further limiting primary production.
- Stand structure is altered rapidly in some areas by salvage logging of high-risk trees; as a result, species composition is changing, and wildlife habitat is being altered.

Oxidant injury to eastern white pine in some forest stands in the eastern United States is a significant problem. There is an important concern about injury caused by a synergistic reaction between ozone and sulfur dioxide at low concentrations.

The relationship between man's welfare and stable natural ecosystems and agroecosystems can be established in terms of the economic and aesthetic values derived from them. In some situations, where ecosystems are stressed by oxidant pollutants, the benefits realized by present and future generations may soon diminish. Considerable research is required to find alternatives that prevent stress or that may salvage some of these benefits. New management strategies should be instituted only when their consequences are predictable within reasonable limits.

EFFECTS OF PHOTOCHEMICAL OXIDANTS ON MATERIALS

In the context of this review, the term "photochemical oxidants" is considered to be synonymous with "ozone." In test chambers that have external ozone generators and that operate at or near atmospheric pressure, ozone is the only likely oxidizing species. In ambient air, however, ground-state atomic oxygen, hydroxyl radicals, and especially hydroperoxy

radicals can compete with ozone in attacking materials, such as rubber, that contain olefinic bonds. The most economically important materials with respect to ozone damage are paint, elastomers (rubber), and textile fiber-dye systems. Damage to polyethylene by ozone is considered to be negligible. The ozone-specific damage in 1970 to materials has been assessed in economic terms and is approximately as follows: paint, $540 million; elastomers, $569 million; and textile fibers and dyes, $84 million. Total material damage attributable to ozone is, therefore, $1.22 billion. This is to be compared with Waddell's estimate of total air pollutant material damage of $2.2 billion. It is clear that ozone is a very important molecule in pollutant damage to materials.

CHAPTER 15.

RECOMMENDATIONS FOR FUTURE RESEARCH

The recommendations for research that appear below is a collage taken from the individual chapters.

CHEMICAL ORIGIN

- Rate constants are needed for almost all the reactions of HO_2 and RO_2.
- The homogeneous and heterogeneous reactions of the oxides of nitrogen with water vapor need study.
- The yields of free radicals from the photolysis of nitrous acid and of aldehydes should be established.
- Equation 4 of Chapter 2, $[O_3] = k_1[NO_2]/k_3[NO]$, should be tested in the real atmosphere, as well as in laboratory experiments. Simultaneous measurements of the concentrations of ozone, nitric oxide, and nitrogen dioxide and of the intensity of sunlight for a variety of conditions will provide a much-needed check on this dynamic equilibrium.
- A quantitative measure of the concentration of free radicals in smog (probably OH or HO_2) under well-defined conditions will provide an important test of present chemical models.
- Strong support for fundamental gas-phase kinetics is needed. Most of the reaction mechanisms and rate constants that are needed to construct realistic and detailed models of the polluted atmosphere are determined in laboratory studies under very special conditions, not in smog simulations at a pressure of 1 atm. Because there are still very serious gaps in the present models, further research should be supported.

- Smog-chamber studies are needed for validating both the detailed chemical models and the lumped models. Many of the past chamber studies have not used sufficiently well-defined initial conditions. Measurements of more products and of the reactive intermediates will provide more stringent tests for models.
- Modeling studies are very useful in pointing out the important kinetic data that are lacking, in clarifying some of the past smog-chamber studies, and generally in making the very complex chemistry more comprehensible. Accurate models can make unique predictions about the polluted atmosphere. There are very useful interactions between the modeling studies, the smog-chamber experiments, and fundamental chemical kinetics; it is not possible to ignore one without hurting progress in the others.
- It seems probable that many new and unstable compounds are present in the polluted atmosphere or in smog chambers. A careful search for some of these compounds may provide some surprises.
- Promising new instrumental techniques should be supported, both for monitoring pollutants and for following reactive intermediates in kinetic studies. A reliable and accurate method of standardizing concentrations in the parts-per-billion range is needed.
- The possibility that free radicals, particularly HO_2, have significant effects on biologic surfaces exposed to the irradiated atmosphere should be investigated. Sticking coefficients are needed. In experiments in which the observed biologic effects cannot be attributed to the measured ozone and PAN concentrations, the possibility of damage by the steady-state concentrations of free radicals in the atmosphere should be considered.

AEROSOLS

Our present knowledge of the chemical and physical processes that govern aerosol formation in the atmosphere is rather limited, and further studies are needed in most of the relevant areas of research. This may leave the reader--and the decision-maker--with a feeling of endlessness. However, substantial improvements could be made in a reasonable period by focusing research efforts in the subjects most directly involved:

- Laboratory (smog-chamber) studies of aerosol formation from aromatic hydrocarbons: gas-phase reaction mechanism, physical processes controlling gas-to-aerosol conversion, kinetic data on aerosol formation and aerosol growth, identification of the aerosol products, and effect of hydrocarbon concentration on aerosol formation (threshold).
- Careful search, in the atmosphere, for aerosol precursors, such as cyclic olefins and C_{6+} alkenes.
- Study of the possible health effects of exposure to difunctional oxygenated organics (such as dicarboxylic acids) that are present in urban aerosols.
- Identification of organic components of ambient aerosols, to permit estimation of the relative importance of olefinic and aromatic hydrocarbons as aerosol precursors.
- Estimation of the relative contributions of photochemical and nonphotochemical pathways to the formation of inorganic nitrate and sulfate aerosols.

Identification of organic components of ambient aerosols and estimation of the contributions of various pathways are of immediate interest for control strategies and could be achieved by using the existing monitoring networks so as to provide more information on aerosol chemical

composition. In view of the adverse effects (e.g., on health and visibility) associated with submicrometer aerosols, an air quality standard for submicrometer particles might be more adequate than the present standard for total suspended particles.

ATMOSPHERIC CONCENTRATIONS OF PHOTOCHEMICAL OXIDANTS

- Nonmethane hydrocarbons and both oxides of nitrogen should be monitored concurrently whenever photochemical oxidant or ozone is monitored.
- Photochemical oxidant monitoring stations should be sited upwind and downwind from urban areas, as well as within those urban areas, wherever possible.
- A common primary calibration standard should be established for all monitoring networks.
- Documentation should be provided in each case to outline the rationale for location and design of monitoring stations and the rationale for data validation for photochemical oxidants.
- A clear indication of what constitutes background concentrations of photochemical oxidants and ozone must be made, in order to form the basis of emission control programs.
- The results of monitoring data must be generalized, in order to relate air quality to emission in a stochastic fashion.

Internal improvements in deterministic methods will be based on accounting for more physicochemical effects in the logical structure. One challenge to the researcher is to do this without making something that is already complex still more difficult to understand, and another challenge is to avoid needless elaboration of detail. Both pitfalls will be avoided, first, by asking how accurate a modeling job is demanded and, second, by carrying out order-of-magnitude analytic appraisals of the omitted phenomenology.

Perhaps the most important thing that research will contribute is a set of criteria delineating the fidelity of existing models, rather than a single supermodel that will consider all effects. Much remains to be done in statistical modeling. The scientific community is on the threshold of potentially great strides with these methods, because of the veritable explosion of data from measurement programs. It is absolutely essential for all agencies interested in environmental management to begin mounting analysis programs that are carefully designed to capitalize on the data base. Traditionally, support has been more readily obtained for making additional measurements in hope that useful information would emerge directly or that someone would spontaneously dig out the useful information. Seldom has either been the case.

Specific research subjects have emerged with respect to improved descriptions of specific phenomena. Some time ago, it was speculated that gas-solid interactions and turbulence effects on reaction kinetics would be important areas of advance in the modeling art. Gas-solid interactions include both chemical formation of aerosols and reactions on surfaces of preexisting suspended particulate matter. Because of differing effects of a material in the gas phase and in some condensed phase, it will be important to characterize transformation processes. The ACHEX (Aerosol CHaracterization EXperiment)

program recently carried out under the direction of Hidy will provide an extensive data base with which to test new ways of treating the gas-solid interaction problem.

The turbulent mixing of emitted reactant gas (such as nitric oxide) with atmospherically formed reactant gas (such as ozone) results in macroscopic heterogeneities, which under some circumstances can significantly change the reaction rate from the value that the mean concentrations used in a rate equation would predict. Airborne measurement from some 40 operational days from the LARPP (Los Angeles Reactive Pollutant Program) study gives 6-s-interval gas-phase data for six gas-phase species simultaneously. This program (under the field management of W. Perkins and under the direction of Coordinating Research Council's CAPA-12 committee, chaired by J. Black) has produced archives of these data that can serve as a test bed for theories of turbulent interactions with kinetics.

In a broader sense, the data obtained from the Regional Air Pollution Study (RAPS) and the California Three-Dimensional Pollutant Gradient Study Program should also serve as bases of further model development. It is incumbent on the agencies responsible for air quality control to identify resources specifically aimed at using these data for improving techniques for designing pollution abatement strategies.

Suggested Applications to Pollution Abatement. Without doubt, the top-priority application of air quality models is the determination of emission controls needed to achieve ambient air quality standards. With the reexamination of transportation control strategies and with the pressures of fuel substitutions, refinements well beyond /

traditional proportional models are imperative. Where validated diffusion models are available, they should be used to recalculate the emission requirements that came from initial hasty efforts to implement the Clean Air Act Amendments of 1970. This is the greatest national service that could be performed by the air quality modelers at present. Before this can be achieved, however, the institutional apparatus must provide the impetus and resources called for in a recent National Academy of Sciences report to the U.S. Senate.

Much of the research work will add content to the model structures, but future applications demand simplifications that are oriented toward the non-specialist user. One of the largest obstacles to the effective use of air quality prediction schemes is the resolution of this apparent conflict. At least two steps can be taken by those who produce models to encourage applications and to aid the user:

- Compile a catalog of air quality models that describes their capabilities in terms of a common set of performance standards.
- Clarify data communication in the input-output interfaces between user and model.

To accomplish the first step, model standards will be evolved on the basis of legislative mandates and regulatory needs. Each of the various types of model has undergone performance evaluation through the application of a set of tests peculiar to its own structure or output. For example, Gaussian models that predict long-term averages are often evaluated by computing only the correlation coefficient between measured and computed concentrations. Early evaluations of species-mass-balance models stressed hour-by-hour comparison of the predicted and observed concentrations. Recently, a broader range of descriptors has evolved, as evidenced by the work of Nappo and Whitney.

The performance indexes must be designed with the model applications in mind. Will the model be used to predict local effects around a highway or a smelter where short-term high doses are as important as long-term averages? Will the model be called on to compare trends in air quality between two different scenarios of urban population growth? Will the model be used to select a control plan that will result in a given hourly air concentration's being exceeded only once a year? A properly designed set of performance standards will allow a potential user to compare models with respect to suitability for any specific application. The particular performance characteristic of interest influences strongly the rank-ordering of models on a scale of goodness.

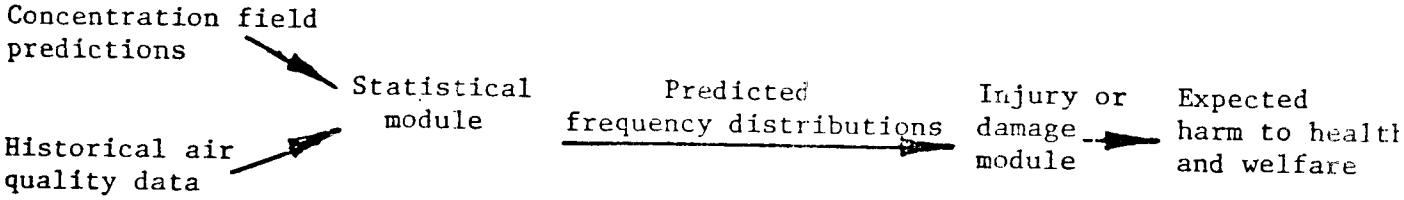
Fundamental to the definition of an optimal set of performance measures will be the relationship of risk (of health, property, or aesthetic attributes) to exposure (average pollutant concentration, time-integrated pollutant concentration, synergistic combination of pollutant dosages, or dosages integrated with respect to space, time, and population). Derived from the risk factor will be, not a single number, but a distribution of effects for each degree of exposure. For example, a range of pulmonary effects can be expected in a sample population in which each individual has been exposed to ozone at an average concentration of 100 $\mu g/m^3$ for 5 years. The expectation value of the effect will be the risk factor that is the function of exposure described above. The model performance index will utilize these relationships to connect a probability density distribution output from the model (associated with imperfect knowledge) to a probability density distribution of the threat to public health and welfare. Stated in a different way, each model will be assessed on the basis of the uncertainty of damage estimate that arises from it imperfections. This must be done in an unambiguous way for the user, who may not be a specialist.

The second step that will be needed to ensure ready application of air quality models is largely a question of packaging and presentation. User-oriented documentation will be needed for personnel at data processing centers, who may not be specialists in chemistry, mathematics, or meteorology. Experience has shown that the user desires to operate the model in his own data center and wishes to understand enough about the model structure to explain it to others in his field. Models that cannot be adapted to these requirements have not been widely applied. In some cases, an operating manual intended for persons with some knowledge of programing will need to be rewritten to allow the user to supply completed data forms to a computer center and routinely receive output in return. Other adaptations may require a user to punch data in on a teletypewriter and receive output on the same machine in an interactive mode. This involves a network of remote terminals served by the computer center, such as that under development in UNAMAP.

Output displays will be required to bring the abstract aspects of voluminous output data into some form that appeals to the experience of the user. Isopleth maps are useful, as are three-dimensional isometric plots like SYMVU, produced by Harvard University. Printer plots of concentration maps will undoubtedly enjoy an even greater application, because of the common availability of line printers or teletypewriters as output devices. Examples of these techniques are SYMAP and GRID, both produced by Harvard University.

Another aspect of matching output to user needs involves presentation of results in a statistical framework--namely, as frequency distributions of concentrations. The output of deterministic models is not directly suited to this task, because it provides a single sample "point" for each run. Analytic linkages can be made between observed frequency distributions and computed

model results. The model output for a particular set of meteorologic conditions can be on the frequency distribution of each station for which observations are available in sufficient sample size. If the model is validated for several different points on the frequency distribution based on today's estimated emission, it can be used to fit a distribution for cases of forecast emission. The fit can be made by relating characteristics of the distribution with a specific set of model predictions. For example, the distribution could be assumed to be log-normal, with a mean and standard deviation each determined by its own function of output concentrations computed for a standardized set of meteorologic conditions. This, in turn, can be linked to some effect on people or property that is defined in terms of the predicted concentration statistics. The diagram below illustrates this process:



We have seen the wide variety of methods now available to calculate air quality. The priority for adapting these methods to current needs is clearly established. Only through clear expositions of model performance and simple implementation procedures will the present techniques have a favorable impact on air quality management. A growing appreciation by the specialist community of the policy requirements will be essential for the successful fulfillment of these goals.

MEASUREMENT METHODS

Instruments based on differential ultraviolet absorption still need to be evaluated, and possibly modified, prior to their acceptance for monitoring ozone in polluted atmospheres on a nationwide scale. The California Air Resources Board and other air pollution control agencies are currently conducting multi-year programs for evaluating ultraviolet absorption side-by-side with chemiluminescent and potassium iodide based instruments, to determine their applicability and needed modification, as well as to assure continuity in the data base while the older monitoring instruments are being replaced.

Thus despite the remarkable progress in the monitoring for ozone, nitrogen oxides and non-methane hydrocarbons, which has strengthened the implementation and evaluation of control programs, substantial research and development is still required to help resolve the uncertainties in our knowledge which are inhibiting the actual achievement of desired air quality standards.

The areas in which further research and development are needed, in sequence of priority are:

- Evaluation of primary calibration procedures applicable nationwide for ozone measurement.
- Development principles and instruments which can easily track the sources of those hydrocarbons reactive in the production of ozone and those which are reactive in the production of particles.
- Chemical identification of both gas and particle phase compounds occurring in the atmosphere, which cause eye irritation and respiratory difficulties.

- Methods for the direct and continual measurement of those chemicals in the particles of the atmospheric haze, that are known to be formed during photochemical pollution episodes and are already suspect as respiratory irritants. By implementing such measurements it will be possible to find out to what extent the occurrence of such substances can be reduced by various emission controls. To assess actual population exposures, it is also necessary that these measurement methods be easily carried out indoors and in vehicles.

- Improved measurement methods suitable for observations from airborne platforms so that the regional scale impacts of urban emissions can be accurately assessed. This is needed because some control options for solving the urban-scale problem have the potential of transferring pollution from one geographic area to other geographic areas.

RESPIRATORY TRANSPORT AND ABSORPTION

The development of models requires more knowledge about the chemical, physical, morphologic, and flow properties of the mucous layer; the kinetics of the reactions of ozone in the mucous and tissue layers; and the molecular diffusivity of ozone in these layers. Similar information is needed for the hydroperoxy, HO_2, and singlet oxygen, O_2 ($a\ ^1\Delta$), free radicals, which are reactive intermediates in photochemical smog.

Furthermore, a realistic model based on such knowledge needs to be verified by measurements of uptake and tissue dosage in the various regions of the respiratory tract. These are currently difficult to make, but are required to establish accuracy and reliability. New methods of sampling and techniques using tagged gases should be developed, so that local uptake can be measured.

An extensive effort is needed in studies of pollutant-gas transfer, absorption, and reaction in the respiratory tract. After some of the experimental questions about behavior of ozone in the mucous layer and adjacent tissue are answered, available methods for calculating the local dosage to critical airway sites can be used in new uptake models. Gas-absorption and particle-deposition models for the upper respiratory tract (nose, mouth, pharynx, larynx) also need to be improved. Experimental data now available can be used to develop semiempirical relations for gas uptake in the nose in a procedure analogous to that used to model particle deposition. Development of a more refined model for nonreactive gases requires data on gas diffusivities in the mucus and tissue, local blood perfusion rates in the nasal epithelium, and physiologic and pharmacologic factors affecting the mucosa and local blood flow rates. Models need to be developed for mixtures of gases that may interact chemically in the gas phase, in the mucus, or in aerosol droplets to form other species. This requires theoretical and experimental studies of dissolution, absorption, adsorption, and desorption of gases in or on aerosols in the respiratory tract.

Improved modeling is needed for the design and interpretation of animal experiments and controlled human studies, and for the collation of diverse data from animal and human exposures to ozone. Calculations of local dose at reactive tissue sites can help to explain the mechanisms of toxicity and are needed to extrapolate animal and human data for assessing population risks under different environmental conditions.

TOXICOLOGY

Enhanced susceptibility to respiratory exposure to infectious agents is of considerable potential public health significance. This has been reported to occur in mice exposed to ozone at as low as 0.08 ppm, the lowest reported "effect" concentration in laboratory animal studies. It seems essential, therefore, to continue and expand research on the effect of ozone and other photochemical oxidants on physiologic protective mechanisms of the lung. Appropriate dose-response studies should be included to confirm or establish the exposure concentration-time relationships that result in increased susceptibility to inhaled microorganisms, and studies of the cellular responses and mechanisms should be conducted with a view to providing methods that are applicable to epidemiologic studies in oxidant-exposed human populations.

Research designed to elucidate the pathophysiologic implications of reversible changes in lung function, histology, and biochemistry that have been observed at concentrations of 0.2-0.5 ppm would be especially valuable in evaluating the significance of these changes for health. In particular, it would be useful to determine what, if any, causal or correlative relationships exist between different effects detected by various experimental assay systems and between reversible changes and more chronic effects, such as reduced lung elasticity, fibrosis, and adenoma formation. Studies on laboratory animals are particularly suited to this type of mechanism-correlative research; but, with appropriate experimental designs, such research should be very useful in determining which methods or biologic changes can be usefully applied to clinical or

epidemiologic studies. At the same time, such research could determine whether some of the changes (e.g., increased activity of enzymes involved in cell redox systems) are indicative of injury or are homeostatic adaptive responses. This information will have value in predicting potential injury in the "average" population and possibly help to identify people who are hypersusceptible by virtue of some deficiency in adaptive protective mechanisms. Related to these research efforts, further investigation of the influence of oxidant exposure on enzymes that biotransform other inhaled chemicals (e.g., aromatic hydrocarbons) and the interaction effect of dietary antioxidants (e.g., vitamin E) should be pursued, particularly in relation to oxidant-induced oxidation of membrane lipids and free-radical formation.

There are several scattered reports of extrapulmonary effects of ozone exposure in laboratory animals. Some (e.g., chromasomal aberrations in hamster lymphocytes) occur at or near concentrations that cause local effects in the lung and portend serious and long-term health implications. Others(e.g., reduction of voluntary activity) may be transient or reversible, but nevertheless contribute to decrements in performance or well-being. It is particularly important that further research be conducted to confirm (or refute) these reported extrapulmonary actions of ozone and the exposure concentrations at which they occur. If confirmation of their occurrence is obtained at or below the currently reported effective exposure concentrations, useful research efforts could be directed toward determining whether they are direct or secondary effects and toward identifying the chemical species responsible. More

importantly, confirmation of an isolated effect on cell genetic material would demand a thorough expert evaluation of its significance on the basis of existing knowledge of the long-term implication of the effect and, accordingly, intensified laboratory and epidemiologic research.

CONTROLLED STUDIES ON HUMANS

Further studies are needed to give better dose-response information and to provide a frequency distribution of the population response to oxidants alone and in combination with other pollutants at various concentrations. Such studies should include the effects of mixed pollutants over ranges corresponding to the ambient atmosphere. With combinations of ozone and sulfur dioxide, the mixture should be carefully characterized to be sure of the effects of trace pollutants on sulfate aerosol formation. The design of such studies should consider the need to use the information for cost-benefit analysis and for extrapolation from animals to humans and from small groups of humans to populations. Recent research has indicated the possibility of human adaptation to chronic exposure to oxidants. Further study is desirable.

Studies are needed to clarify the importance of age, sex, ethnicity, familial elements, nutritional factors, and pharmacologic agents in determining response to oxidants. Because people with lung disease are thought to be more susceptible to oxidant pollutants, exposure studies are needed to quantify this. Better methods for measuring or estimating the actual dose of oxidants absorbed by each subject are needed. The usual time variation in measures of human response should be evaluated per se, because this information is needed to optimize experimental design.

More information is needed before rational guidance can be given about limiting exercise during periods of high oxidant pollution.

Standards for the exposures of humans to controlled atmospheres should be discussed by national groups and agencies, such as the National Academy of Sciences, the American Medical Association, and the National Institutes of Health.

EPIDEMIOLOGIC STUDIES

Modification of the method in which the CHESS studies are designed and in which the data are displayed would add considerably to their value. The scientific data collected in all the CHESS studies should be made available through accepted scientific publications to the scientific community as a whole.

The continuation of epidemiologic studies, including those of the CHESS program, is vital to our understanding of the effects of air pollution on health. There is no other way to determine the needed dose-response (exposure-response) relationships between the complex urban atmospheres and specific health effects. No animal or clinical experiment can duplicate the full range of variables to be found in ambient urban air. For this reason, all necessary support should be given to qualified scientists to conduct epidemiologic studies designed to answer these questions. Although such studies are expensive and time-consuming, the data they produce can be produced in no other way and are essential in the development of useful air quality standards.

It is important to know whether such phenomena as tolerance and cross-protection, well demonstrated or suggested in animals, occur in man. Analogues for all these phenomena should be sought in human populations, and methods should be devised for assessing their significance for human health.

PLANT AND MICROORGANISMS

These recommendations are not listed in a priority order, but many could be followed in parallel or simultaneously. In general, the recommendations presented by Heck et al. are still germane to research needs in the subject of oxidant pollutants.

A few definitive experimental designs are needed to further our knowledge of acute dose-response information on ozone. Much of this type of information is still needed for PAN and its analogues. All experimental designs should incorporate dose response.

Studies to develop dose-response curves for chronic exposures of crop and native species over growing seasons and under field conditions are needed for ozone, PAN, and other oxidants.

Research should be continued with filtered and nonfiltered field chambers to study effects of ambient oxidants on important agronomic, horticultural, and native species. We know that there is a problem, but its significance and magnitude are matters of conjecture.

There is a critical need to understand the interaction of multiple pollutants on individual plant species and ecosystems. Multiple-pollutant effects are generally important, but little is known of their effects on most plants. Variable concentrations, ratios of pollutants, and age of plants all affect response.

Models should be developed to understand the relative importance of other variables as they affect plant dose response. These include, but are

not limited to, climatic, edaphic, biotic, and genetic factors. Considerable information is available, but there are many gaps, and no comprehensive programs are in progress to determine how these factors act and interact to affect a plant's response.

The mechanism of response and the biochemical systems affected are not understood. Although plant membranes are considered the primary sites of action for the oxidant pollutants, there is no definitive work on this. An understanding of these responses would be supportive of breeding and spray protective programs.

Both breeding and spray protective programs need to be developed, so that better-yielding and better-quality cultivars will be protected against oxidant pollutants.

Some effort is needed to explore the feasibility of using plants to monitor the overall biologic activity (or biomass reductions) caused by photochemical oxidants in specific air basins or regions. The response of sensitive plants should be correlated with the response of plants of economic and aesthetic importance.

Additional monitoring of multiple pollutants is needed in rural areas.

Whenever possible, measures that vary on a continuous scale (e.g., biomass) should be used with subjective estimates of injury (e.g., indexes of visible injury).

ECOSYSTEMS

The present knowledge of the biologic consequences of chronic oxidant injury to both natural ecosystems and agroecosystems must be communicated to groups in the public sector that work in planning and enforcement. The indirect effects on man's health and the direct effects on his welfare resulting from ecosystem deterioration due to oxidant injury are serious

enough to be given more thorough consideration in all decisions related to abatement of air pollution from both mobile and stationary sources. Land-use planning and proper airshed classification should be used to prevent further deterioration of air quality, particularly in prime timber-producing areas and in remote, pristine areas, regardless of their present use designation.

The most important research needs are related to the determination of the responses of natural ecosystems and agroecosystems to chronic exposure to oxidant pollutants. In particular, chronic-dose-response models are needed to understand the responses of the dominant primary-producer species constituting forest ecosystems in both the eastern and the western United States. The resulting alteration of interactions with other subsystems--e.g., consumers and decomposers--must also be investigated.

EFFECTS OF PHOTOCHEMICAL OXIDANTS ON MATERIALS

- Laboratory studies of effects of photochemical oxidants other than ozone--e.g., PAN, peroxybenzoylnitrate, atomic oxygen, excited molecular oxygen, $O_2(^1\Delta_g)$, and hydroperoxy and hydroxyl radicals--on specific materials should be conducted:
- **Methods to measure concentrations of transient oxidants such as OH, HO_2, O_2 (^1O_g) in real atmospheres should be developed.**
- Mechanisms of attack of oxidants on materials should be investigated, so that distinctions can be made between effects on a given material due to various pollutants.
- An integrated study should be conducted in which relative effects of all major air pollutants on materials are assessed.