

# Risk Assessment of Oxidant Gases and Particulate Air Pollutants: Uncertainties and Research Needs

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The assessment of risks to human health associated with exposure to oxidant air pollutants has not received adequate attention despite the recognized public health threat posed by the ubiquitous presence of these compounds in the environment. In this article, research needs and uncertainties at each of the steps in the risk assessment of oxidant air pollutants are identified: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Many of these limitations and uncertainties arise at the interface between the laboratory and the regulatory arenas. Therefore, as a case study, relevant methodologic problems associated with the application of experimental findings to the risk assessment of respirable dusts are also discussed. These issues include the extrapolation of animal data to the human case and extrapolation from high-dose to environmentally relevant, low-level exposures. — Environ Health Perspect 102(Suppl 10):209–214 (1994)

Key words: risk assessment, air pollutants, particles, oxidants

## Introduction

A significant part of the population is routinely exposed to ambient air pollutants that have oxidizing properties. These ambient oxidants include photochemical oxidant pollutants, such as ozone (O<sub>3</sub>) (1–3) and the nitrogen oxides NO<sub>2</sub> and NO (NO<sub>x</sub>) (4), as well as certain mineral dusts that can act in part by oxidant mechanisms, such as crystalline silica (5,6) and asbestos (7). The ubiquitous nature of ambient oxidants in the environment is underscored by estimations that over 100 million Americans are exposed to levels of O<sub>3</sub> that exceed the current National Ambient Air Quality Standard (8), and

that a normal human lung contains up to two million asbestos fibers per gram of dry tissue (9,10).

Numerous toxicologic and epidemiologic studies have demonstrated adverse health effects as a consequence of exposure to oxidant pollutants at environmentally relevant concentrations, and the presence of oxidant pollutants in the troposphere has long been recognized as a public health concern. However, there have been few studies directly aimed at improving the assessment of risks that these compounds pose to human health. In this article we outline the process of risk assessment as it is currently practiced by regulatory agencies in the United States. We also identify specific areas of investigation where additional information is needed to undertake studies on the health risks presented by exposure to oxidant pollutants found in ambient air. As a case study, relevant methodologic problems in the application of laboratory findings to risk assessment are then illustrated in a discussion of the toxicology of respirable dusts.

## The Risk Assessment Process

Risk assessment is a process that leads to an estimation of the probability that an untoward effect will result from exposure to a substance given a defined set of circum-

stances. Although the terms are sometimes used interchangeably, risk assessment is a process distinct from that of risk management, the latter uses the results of a risk assessment in the development of policies aimed at identifying and reducing risks.

The paradigm for risk assessment that is most widely used in the United States was first put forth by a 1983 National Research Council report that has come to be known as the “Red Book” (11). Essentially, the process consists of four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization.

As the initial step, hazard identification establishes a preliminary causative link between exposure to a chemical and a specific deleterious effect on health. The types of scientific information used in a hazard identification include epidemiologic, animal toxicity, and *in vitro* toxicity data. In addition, findings derived from structure-activity relationships and computer modeling sometimes are used.

What is sought in conducting a dose-response assessment is a description of the quantitative relationship that exists between exposure to a compound and the nature, incidence, or severity of a specific toxic outcome of that exposure. Data for this step typically are drawn from two sources: experimental animal studies and, preferably

This paper was presented at the Conference on Oxygen Radicals and Lung Injury held 30 August–2 September 1993 in Morgantown, West Virginia.

JMS was supported by a 1993 American Association for the Advancement of Science/U.S. EPA Environmental Science and Engineering Fellowship.

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but more rarely, human data derived from accidental, occupational, or controlled human exposures. It is worth emphasizing that separate dose–response assessments are needed for each end point of toxicity. These data evaluations then are used as a basis for the various extrapolations and assumptions that risk assessors have to make when faced with significant gaps in the knowledge base of the adverse effects of the chemical/physical agent in question. This facet of the dose–response assessment is often the most contentious step in the risk assessment process.

Exposure assessment concerns the identification and characterization of the populations exposed and determines the magnitude and duration of the exposure. This step relies on demographic analysis and environmental monitoring and is often the most neglected aspect of risk assessment, mainly because often much of the information needed is unavailable. Finally, in the risk characterization phase, the results of the first three steps are integrated to produce an estimate of the likelihood that an adverse health effect will occur, and the frequency and severity with which the outcome can be expected in the population under specified conditions.

### Issues in the Risk Assessment of Oxidants

A pivotal part of any hazard assessment is the identification of specific and relevant end points of toxicity. In that regard, the hazard identification for most ambient oxidant pollutants has in part already been done, since untoward effects have already been associated with exposure to these agents. Because the principal route of exposure to oxidant pollutants is inhalation, the respiratory tract is the most relevant target of their toxicity. However, other sources such as ingestion or dermal exposure also could be important. The pulmonary toxicology end points produced by oxidant exposure can be categorized as those that result from acute exposure and those produced by chronic exposure.

A list of the acute end points would include: pulmonary function changes, pulmonary edema, the presence of inflammatory cells or inflammatory mediators in bronchoalveolar lavage fluid (BALF), changes in the content of antioxidants in BALF, evidence of genetic damage that may be apparent in lung cells obtained by lavage, biomarkers of oxidant damage such as pentane and aldehydes in exhaled breath, effects on host defense mechanisms, and changes in the redox status of lung

cells that may provide evidence of accelerated aging or “toxic senescence” in the lung. This list is not complete and other end points could well be added to it (12).

End points of chronic exposure could include all of the changes under the acute category in addition to alterations in lung morphology, and other parameters that may suggest obstructive, restrictive, or neoplastic disease in the lung as a consequence of exposure to ambient oxidants. The hazard identification of oxidant pollutants would be greatly facilitated if one or more end points of toxicity common to all environmental oxidants could be identified, since this would make it possible to treat oxidants as a class of compounds and possibly permit ranking them according to their potency.

The dose–response assessment for a specific toxic end point that results from exposure to an ambient oxidant would be the next step undertaken in a risk assessment study. Fortunately, many of the oxidants are relatively well-studied compounds. For certain oxidants such as  $O_3$ , a substantial amount of human data at environmentally relevant concentrations already exists. These data are derived from studies of occupational exposures in the case of asbestos and from controlled human exposure studies in the case of  $O_3$  and  $NO_x$ . There are also a significant amount of animal data, including findings from chronic exposures to some oxidants. In addition, the recent introduction of techniques that use nonradioactive isotopes of oxygen promises to elucidate the relationship that exists between exposure concentration and the dose that actually is delivered to the relevant parts of the respiratory tract (13). In spite of this progress, however, there are also several unresolved issues surrounding the dose–response assessment of oxidants. For example, if a common end point of toxicity can be found for some oxidants, can a dose equivalency for exposure to them be calculated? If so, is the redox potential of an oxidant predictive of its potency as an oxidant? And last, how should we deal with oxidant injury caused by inflammatory cells attracted to the site of injury by the initial oxidant insult to the lung?

The exposure assessment of many oxidant pollutants also presents challenges. For example, epidemiologic evidence suggests that certain segments of the population are at a higher risk of injury by exposure to ambient oxidants; for example, the elderly (14), children (15), and people with existing pulmonary conditions such as

asthma (16) or respiratory allergies (17). More data on the nature of the exposure of these and other segments of the population that might be especially susceptible are needed. The role of dietary factors, specifically dietary antioxidants, needs elucidation in view of epidemiologic studies that show an increased risk of various types of cancer associated with antioxidant vitamin deficiencies (18,19). Another issue that has been discussed is the appropriateness of the current monitoring and regulatory strategies. Currently exposure limits for pollutants such as  $O_3$  are given as a “maximum one-hour exposure level.” However, results from animal exposure studies argue in favor of using an approach that also takes into account cumulative exposure and the episodic nature of environmental exposure to air pollutants (20).

Areas of research that would produce information applicable to all stages of the risk assessment of oxidant pollutants can be categorized only in broad terms here. More data on basic mechanisms of inflammation in the lung would be helpful for two reasons. First, much additional information is needed before it is possible to distinguish between oxidant injury that is produced by exposure to oxidant compounds and that which is caused by inflammatory cells migrating to the site of injury. Second, current information indicates that some type of inflammatory response is an end point common to the pulmonary toxicity of most, if not all, of the ambient oxidants.

Reliable biomarkers of exposure to oxidants are also needed to facilitate the dose–response and exposure assessments. For obvious reasons, noninvasive biomarkers, such as markers of lipid peroxidation (e.g., pentane) found in exhaled breath, are the preferred type. Finally, much more information is needed on the effects of chronic exposure to low, environmentally relevant concentrations of oxidant, given that this pattern of exposure describes that which affects large segments of the population. In particular, the effects of repeated or sustained exposures to low levels of oxidants on lung connective tissue and the possible role of oxidants as carcinogens or tumor promoters needs elucidation.

### Pulmonary Toxicology of Inhaled Particles: Lessons for Risk Assessment

The process of risk assessment described in the preceding paragraphs poses a number of problems, some of which can be summarized with the following questions. First,

for a given material or toxic response, which animal species is most appropriate for extrapolation to human health risk assessment? Second, given that most exposure studies are performed in healthy inbred animals, how relevant are these results to predicting effects in humans who show wide genetic variability and may have compromised health? Third, how appropriate is the assumption that the mechanisms leading to a specific toxic response in experimental animals are the same in humans? Fourth, which mathematic model should we use to address the crucial issues of high-to-low exposure and animal-to-human extrapolations? In this section, the example of pulmonary effects of inhaled particulate compounds will be used to discuss these questions.

Recent epidemiologic studies suggest that levels of total suspended particle concentrations at or below the current National Ambient Air Quality Standard of  $150 \mu\text{g}/\text{m}^3$  cause increased morbidity and mortality in humans. There is evidence that these effects occur at particle levels as low as  $50 \mu\text{g}/\text{m}^3$ , a concentration that does not appear to have any effect in laboratory animals. A key question is whether this is a real effect that can be causally connected to ambient particle air concentrations. While the correlation between morbidity/mortality and exposure to airborne particles may be clearly established from epidemiologic studies (21,22), such evidence can not prove causality. Effects of inhaled particulate compounds in animals have been demonstrated to occur with exposure to much higher concentrations, in particular if highly insoluble particles of low intrinsic toxicity are considered. Those particles, formerly characterized as "nuisance" particles, can lead to acute as well as chronic effects when the inhaled concentrations are in the several  $\text{mg}/\text{m}^3$  range, i.e., orders of magnitude higher than those encountered under human environmental exposure conditions. Chronic effects observed in such high exposure animal studies (rats appear to be especially sensitive) include lung inflammation, fibrosis, and even lung tumors (23).

In animal studies it was consistently found that at excessive exposure concentrations the particle clearance function of alveolar macrophages is severely impaired. Retardation of clearance appears to be due to "overloading" of the macrophages with phagocytized particles, thus the term "particle overload" has been used to describe this situation (24). The significantly retarded particle clearance results in

increased accumulation of particles in the lung. Fibrosis and lung tumors frequently develop in rats after such excessive exposure to particles. In contrast, in other species, such as hamsters and mice, the same high exposures produce lower incidences of inflammation and relatively few fibrotic and no neoplastic lesions in comparison to rats (23,25,26).

This raises the important question as to which animal model is the most relevant for extrapolation to humans. Although humans exposed to high particle concentrations (e.g., coal miners) also show inflammatory and fibrotic responses in their lungs, no increased lung tumor incidences have been observed in this particular population. Unfortunately, few studies of the respiratory effects of coal dust on rats have been performed. The question then becomes: Can the results from high-dose exposure studies in rats be used for extrapolation to humans, or should we restrict such extrapolations to specific mechanistic effects that can also be demonstrated in other species? For example, an impairment of alveolar macrophage-mediated particle clearance has been observed in all experimental species exposed to high concentrations of particles and may therefore be considered likely to also occur in humans.

This brings us to the third question that was listed earlier—the influence of health status on particle-induced toxicity. Animal studies generally are conducted in healthy, specific pathogen-free rats, mice, and hamsters that are bred and kept under conditions that protect them from any environmental hazard other than the specific exposures being studied. In contrast, epidemiologic studies showing a correlation between very low ambient air particle levels and increased mortality/morbidity in humans also show that the effects are limited to individuals with compromised cardiorespiratory function. Thus, dose-response relationships observed in healthy animals are not likely to be applicable and extrapolatable to potentially more susceptible humans, and we could be searching for answers using the wrong animal model.

The impact of particle size also needs to be considered since several studies have shown that particles in the so-called ultrafine particle range ( $< \sim 100 \text{ nm}$ ) have a significantly greater adverse effect on the lungs than larger particles (27). Ultrafine particles are present in the ambient air and their concentrations appear to be greater under certain meteorologic conditions

(Castellani, personal communication) and may therefore contribute to the observed epidemiologic findings.

The focus of toxicologic studies on ultrafine and larger particles has been mainly on chronic effects; there is also evidence that freshly generated polymer fumes consisting of ultrafine particles can cause severe acute effects, including lethality.

Paradoxically, the acute effects of freshly generated fumes can occur at particle concentrations that are much lower than those used in chronic particle inhalation studies performed in animals (28). These effects have been attributed to the ultrafine particle phase rather than to the gas phase of polymer fumes, and they indicate that ultrafine particles may cause severe acute effects in the lung. While knowledge of the potential toxicity of ultrafine particles is not sufficient to explain the epidemiologic findings in humans, it could form the basis for the design of more detailed studies.

The paramount questions to be addressed when trying to assess human risks based on animal studies pertain to the mechanistic events underlying the observed effects. Are such mechanisms likely to be the same in animals and humans? With respect to particles, several investigators have shown that the interactions of particles with inflammatory cells in the lung lead to the production of inflammatory mediators, cytokines, and especially reactive oxidant species that in turn may be responsible for adverse effects on other lung cells (29). Some particles have additional toxic properties imparted by reactive groups on their surfaces (30,31). There are a number of antioxidants (e.g., glutathione, ascorbic acid,  $\alpha$ -tocopherol, metallothionein) present in different lung cells that counteract the effects of oxidants in the lung; however, antioxidant levels in different animal species vary considerably (32–34). Interspecies variability in lung antioxidant concentrations therefore could be responsible for the differences in susceptibility observed in different animal models. In addition, the inducibility of antioxidant systems in response to prior exposure to oxidants may also be significantly different, the mouse being more responsive than the rat, for example (33). Differences in other metabolic pathways, such as cytochrome P450 monooxygenases, may also lead to differences in susceptibility to particle-induced toxicity in the lung. These factors taken together may contribute to the greater resistance to particle toxicity in one animal species versus

another, and need to be considered when comparing species and attempting to extrapolate to humans.

Finally, the question of the mathematic model to be used in extrapolation is a central issue in the risk assessment process. With respect to the above-mentioned carcinogenic effects of inhaled particles in rats, the linearized multistage model is generally used by the U.S. EPA to define a cancer risk (35). For inhaled particles, the unit risk for carcinogenesis is the 95% upper-bound of the estimated risk from continuous lifetime exposure to an airborne compound at a level of  $1 \mu\text{g}/\text{m}^3$ . However, in the case of lung tumors resulting from particle overload, it can be argued that we may be dealing with a threshold effect, i.e., below a certain lung dose, no such effects might be expected. As an example scenario, where particles of low solubility and inherent toxicity and the clearance of the deposited particles is not impaired, one would not expect to see any adverse long-term effects since no excessive accumulation of particles in the lung would occur. Indeed, long-term animal studies confirm that when pulmonary clearance is not dis-

turbed by particle exposure, no adverse pulmonary effects occur, thereby arguing for the threshold model of extrapolation. Should these findings be considered when choosing an extrapolation model, or should we assume that humans do not have a threshold in their response to particle inhalation?

On the other hand, as mentioned previously, ultrafine particles exert their effects at a lower concentration and are more toxic at the same concentration than larger particles of the same composition, e.g., titanium dioxide (27). If thresholds exist for particle-induced effects, do they disappear at sufficiently small particle size? Types of toxic effects may also differ with particle size. In assessing human risk, it is also necessary to consider that most individuals have a preexisting particle burden. In some cases, heavy smokers, persons exposed to dusty environments, etc., may have sufficient particle loads to inhibit clearance (36,37). Even if a threshold for toxic effects exists, assessment of risk based upon a threshold would be inappropriate for such individuals. It is therefore apparent that many uncertainties exist in the low-

dose extrapolation of risk from particle exposure.

Additional questions can be raised as to how particles should be tested in animal studies. Specifically, what should be the highest dose to which rats or other laboratory animals are exposed in a chronic inhalation study? The concept of maximum tolerated dose (23,38) may need to be redefined and expanded to incorporate the "overload" effect in the decision making process towards the setting of air quality standards at the workplace.

This brief discussion, based mainly on the results of animal studies, has focused on only a few of the many questions related to risk assessment, yet it shows the difficulties and uncertainties surrounding the process. Clearly, the more mechanistic data that can be obtained for a particular inhaled toxicant, the better our capabilities to extrapolate animal data to humans and, therefore, a major emphasis in the risk assessment of oxidant pollutants should be placed on the elucidation of their mechanism of toxicity.

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