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Interaction of gaseous and particulate pollutants in the respiratory tract: mechanisms and modulators

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Abstract

Human contact with air pollution usually involves exposure to more than one chemical, and biological responses to the inhalation of polluted atmospheres likely depend upon the interplay between individual materials. Thus, characterizing effects from exposures to mixtures of air pollutants is necessary for adequate quantitation of health risks. Exposure to gas/particle mixtures may result in respiratory tract responses which are additive, or reflect synergistic or antagonistic interactions. The occurrence and type of interaction depends upon numerous factors, including the biological endpoint being examined and the specific exposure conditions, such as concentration, duration, and the physicochemical characteristics of the exposure atmosphere. It is, therefore, not always possible to predict solely from the presence of certain pollutants in a complex atmosphere exactly whether there will be an interaction and, if so, what type it will be. This complicates attempts to relate responses observed in laboratory studies of mixtures to those which may occur under ambient patterns of exposure, an extrapolation needed for human risk assessment.

Keywords: Air pollution; Risk assessment; Chemical mixtures

1. Introduction

The surfaces lining the respiratory tract are in direct contact with the external environment, in the form of about 10 000 l of air which are inhaled daily. This air often contains numerous potentially hazardous gases and particles derived from multiple sources. Thus, exposure to polluted ambient air represents a real problem in interpreting the toxicity of mixtures that are both chemically and physically complex. But while human contact with air pollution usually involves exposure to more than one chemical, and biological responses to the inhalation of polluted atmospheres likely depend upon the interplay between individual materials, experimental studies have routinely examined effects resulting from single pollutants. Because of this more readily available database for individual chemical exposures, public health-based air quality standards have generally been set without regard for potential interactions between the materials being regulated. Thus, char-

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acterization of effects from exposures to relevant mixtures of air pollutants is necessary for adequate quantitation of health risks.

This paper provides an overview of interactions which may occur between gases and particles found in polluted air, based upon controlled toxicological studies in animals and, in some cases, in vitro exposures. It is not intended to be exhaustive but, rather, outlines mechanisms likely underlying such interactions in relation to the respiratory tract, and factors which influence their occurrence or nature.

2. Definition of terms

Since the terminology of interaction is often quite confusing, any discussion must clearly define the specific terms used. Thus, for current purposes, interaction is defined as occurring when the inhalation of two or more chemical agents results in a biological response which differs from that expected based upon responses to the same agents if inhaled singly. These differences may be qualitative or quantitative in nature; the former is important, since interactions may result in quite unexpected biological responses based upon known actions of the individual chemicals. Furthermore, interactions may occur with simultaneous or sequential exposures, as will be discussed. If the lack of interaction is considered to be additivity, i.e. the combined effect of the agents is the sum of their individual effects, then synergism occurs when the effects of the agents are greater than additive, and antagonism when the effects are less than additive. To add to the complexity of the terminology, synergism can also be considered as the case where one chemical has an effect and another does not, but the combination increases the response to the former, or the case where none of the mixture constituents has an effect when given alone, but exposure to the mixture produces some response. When considering this last situation, it is important to have some knowledge of the dose-response curve for each individual agent, since the finding of an effect with the mixture may merely reflect additivity, bringing the summed responses to the agents as components of a mixture above the threshold level for a measurable effect.

3. Approaches for assessing gas/particle interaction in the respiratory tract

A number of approaches have been used to examine responses to mixtures in relation to respiratory tract injury. Perhaps the most realistic one is to expose animals to actual ambient pollution. Bils (1966) exposed mice for varying time periods to Los Angeles ambient air, and noted histological changes in the lungs, including loss of surfactant. More recently, mice exposed to Los Angeles air showed increased number and area of Type 2 pulmonary cells, which may be an index of early lung injury, compared to animals exposed to the cleaner air of Santa Barbara (Sherwin and Richters, 1991).

Rats exposed for 6 months to the ambient atmosphere of São Paulo, Brazil showed bronchial secretory cell hyperplasia, increased mucous secretion and decreased mucociliary function; these changes were not observed in rats exposed to cleaner rural air (Saldiva et al., 1992). Finally, Böhm et al. (1989) exposed rats to the ambient air of São Paulo and Cubatao, Brazil; pollution in the former appeared to be dominated by automobile exhaust gases, while that in the latter by industrially derived particulate matter. Rats exposed in Cubatao showed histopathological responses, such as mucus hypersecretion and epithelial hyperplasia, in both the upper and lower bronchial tree, while those exposed in São Paulo showed effects generally limited to the upper bronchial tree. The higher particle concentrations in Cubatao were suggested to be responsible for the differential effects.

The above studies are, in a sense, the toxicological equivalent of epidemiology, and while they may indicate that daily exposure to 'real' ambient air mixtures can result in adverse pulmonary effects, the role of specific particles or gases, and combinations thereof, in producing these cannot always be determined due to the presence of varying confounding factors. On the other hand, controlled toxicological investigations can show that specific combinations of ambient pollutants are associated with interactive toxicological effects. Most of these studies involved exposure to simple, or binary, mixtures, i.e. one gaseous and

one particulate component. However, the pollutant atmosphere in most environments is a complex mixture of a number of agents, and assessing effects of multicomponent atmospheres should serve to provide some indication of biological responses under conditions which better mimic ambient exposures. Even though these studies may be performed under controlled conditions, the results are often difficult to interpret due to chemical interactions between mixture components, the production of variable amounts of numerous secondary reaction products, and a resultant lack of precise control over the actual chemical composition of the inhaled atmosphere.

4. Mechanisms of interaction

The mechanisms underlying specific interactions are not always clear. However, various hypotheses have been proposed, and these are discussed below, along with some likely examples of each.

4.1. Physical adsorption

The basis for many interactions is physical, the result of adsorption of a gas onto the surface of a particle and subsequent transport within the respiratory tract to more 'sensitive' target sites, or to sites where the gas would not normally deposit in sufficient amounts to be hazardous. This mechanism is especially important for those gases that, if inhaled alone, would normally be scrubbed to a large extent in the upper respiratory tract (head airways), but by being carried on particle surfaces are able to penetrate this region and become available for deposition within the lungs.

An early study examined this mechanism of interaction for enhancing the toxicity of nitrogen dioxide (NO_2), a gas which normally shows substantial uptake, up to 90% of the amount inhaled (Schlesinger, 1992), within the upper respiratory tract. Boren (1964) exposed mice (6 h/day, 5 days/week for 3 months) to carbon particles onto which nitrogen dioxide was adsorbed (553 mg NO_2/g C). The animals thus exposed showed focal parenchymal lesions containing carbon, and which were characterized by enlarged air spaces and loss of alveolar septa. Since exposure solely to nitrogen dioxide resulted in edema and inflammation, but no parenchymal lesions, while no effects were noted with exposure solely to carbon, it was concluded that the particles served as carriers for nitrogen dioxide, delivering high concentrations of this irritant gas to localized areas within the lungs where the particles deposited. This was, in effect, an early example of synergism.

Carbon can be considered as an example of an 'inert' particle, which has little effect when inhaled alone. However, adsorption of gases may increase the toxicity from particles which themselves may produce adverse effects. Thus, for example, Shevchenko (1971) chronically exposed rats to quartz, a fibrogenic particle, both with and without adsorbed nitrogen dioxide (0.36 mg NO_2/g quartz). An increase in the fibrogenicity of the particles was found in the animals exposed to the mixture, compared to those exposed to the pure particle, again suggesting synergism resulting from gas adsorption onto particle surfaces.

Adsorption may also underlie interactions between carbon and certain organic gases, namely the aldehydes, formaldehyde and acrolein. Because of their high aqueous solubility, a large fraction of these gases would normally deposit within the upper respiratory tract — $>95\%$ for formaldehyde and about 80% for acrolein (Aharonson et al., 1974; Leikauf, 1992). Jakab (1992) exposed mice (4 h/day for 4 days) to mixtures of formaldehyde (5 ppm) and carbon black (10 mg/m^3, 2.4 μm). The phagocytic activity of pulmonary macrophages was reduced following exposure to the mixture, while no effect was noted with either the carbon or formaldelyde when given alone.

In a related study (Jakob, 1993), mice were exposed (4 h/day for 4 days) to mixtures of acrolein (2.5 ppm) and carbon black (10 mg/m^3), and changes in infectivity to four microbes, *S. aureus*, *P. mirabilis*, *L. monocytogenes* and influenza A virus, administered 1 day following the last exposure, were assessed. The mixture reduced both pulmonary elimination of the virus and intrapulmonary killing of *L. monocytogenes*, effects not seen with the pollutants when given alone. Intrapulmonary killing of *S. aureus* was also reduced, but that for *P. mirabilis* was increased by

the mixture; carbon alone had no effect. These results indicated interaction, likely synergism, with exposure to mixtures of carbon and acrolein and, since the microbial agents were selected on the basis of the defense mechanisms they elicited, also suggested that various aspects of pulmonary defense were differentially affected. For example, since the responses with *L. monocytogenes* and influenza virus were more persistent than with the other two microbes, it was suggested that the particle/gas mixture had a greater impact upon acquired immune defenses underlying protection against these two microbes than on innate, nonspecific defenses mediated by macrophages and neutrophils responsible for protection against *S. aureus* and *P. mirabilis*.

A gaseous pollutant common in ambient air is ozone (O_3), and even though it is not highly soluble in water it is very reactive and, when inhaled, will show 40–50% removal in the upper respiratory tract (Gerrity, 1989; Hatch et al., 1989). When mice were exposed (4 h) to a mixture of ozone (1.5 ppm) and carbon black (10 mg/m^3, 2.4 μm), a reduction in the phagocytic activity of pulmonary macrophages recovered by lavage, and an increase in the number of neutrophils, were noted; the effects were greater than those found when ozone was inhaled alone, and carbon alone had no effect at all, suggestive of synergism (Jakab and Hemenway, 1994). These responses may have been due to adsorption of ozone onto the particle surface and subsequent transport to the lower respiratory tract at a higher concentration than if the ozone was inhaled alone. On the other hand, they may reflect the production of some toxic intermediates formed on the particle surface, as discussed below. Thus, the mechanistic basis for interaction cannot always be elucidated with certainty.

The role of carrier particles in transporting chemicals into the respiratory tract has been of great interest as one basis of carcinogenesis related to the inhalation of complex atmospheres derived from combustion processes, e.g. diesel exhaust. Such processes produce various organic carcinogens, such as polycyclic aromatic hydrocarbons, and these become associated with carbon particles also found in the combustion effluent. One manner by which interaction may be manifested is a change in the bioavailability of the organic compounds within the lungs if they are inhaled adsorbed to particles, compared to if they are administered as a pure compound. This can occur by altering the respiratory tract deposition and clearance of the organics which may then, in turn, alter their carcinogenic potential. For example, a greater pulmonary retention of benzo[*a*]pyrene and greater extent of reaction with lung macromolecules occurred when this hydrocarbon was adsorbed onto carbon particles than when exposure was to the free organic material (Sun et al., 1989). In addition, more lung tumors seem to develop following exposure to particle-bound polycyclic aromatic hydrocarbons than following exposure to the organic material alone (Lindenschmidt and Witschi, 1990).

An important factor to be considered in assessing carrier particle/gas interactions is the rate of desorption or elution of the adsorbed material from the particle. Thus, for example, if an organic material is inhaled on a particle having a slow rate of physical clearance from the respiratory tract, interaction may not occur if the adsorbed material is eluted slowly from the carrier, since particle translocation from the respiratory tract may occur before the dose from the desorbing agent is high enough to produce any toxicity.

4.2. Chemical reaction in the exposure atmosphere or on a particle surface

Gas-particle interactions may reflect the secondary production of chemical species following reaction between the primary constituents of mixtures before, or after, inhalation. One of the earliest documented, at least anecdotally, examples of the former occurred during the famous London 'killer fog' episode of December 1952. During this time, cattle which were housed at a livestock show developed acute respiratory symptoms, while pigs and sheep at local farms were not affected (Waldbott, 1973). Sulfuric acid (H_2SO_4) was concluded to be one of the pollutants likely responsible for the increased mortality and morbidity in the human population during this pollution episode (United Kingdom Ministry of Health, 1954), and the lack of response of the local animals probably

resulted from their being housed in pens that were not regularly or well cleaned and, therefore, contained high levels of ammonia (NH_3), compared to the well-groomed housing areas of the cows held at the livestock show. The reaction of ammonia with sulfuric acid would have produced ammonium bisulfate (NH_4HSO_4) and ammonium sulfate [$(NH_4)_2SO_4$] in the ambient air, and both of these are less potent respiratory irritants than is sulfuric acid. Thus, in this case, chemical reaction in the exposure air resulted in a mitigation of the toxicity of the particulate components of a mixture, i.e. antagonism.

In a laboratory study, Pattle et al. (1956) noted that if sufficient ammonium carbonate was added into a chamber in which guinea pigs were exposed to sulfuric acid particles so as to result in the release of excess ammonia gas, protection was afforded to acid levels that, in the absence of ammonia, would have been lethal to the animals. This is another example of chemical reaction in the exposure atmosphere reducing the toxicity of one component of a mixture.

Mitigation of the effects of inhaled sulfuric acid can also occur by reaction with endogenous ammonia within the respiratory tract. For example, Schlesinger and Chen (1994) noted that when sulfuric acid or ammonium bisulfate were inhaled from atmospheres having the same concentration of hydrogen ion, the effects of the former were greater than that of the latter. This was ascribed to differential neutralization by respiratory tract ammonia, resulting in a greater reduction of hydrogen ion (H^+) content following inhalation of ammonium bisulfate than sulfuric acid, and a reduced response with the former.

The interactions described above all resulted in a reduction of toxicity from what would be expected of constituents of the inhaled exposure atmosphere, resulting in antagonism. However, interactions may also result in a greater response compared to that anticipated based upon what is known about the toxicity of the primary constituents of an inhaled mixture, producing synergism. For example, Kleinman et al. (1989) exposed rats (4 h) to a complex mixture atmosphere, designed to represent photochemical pollution, which consisted of ozone (0.6 ppm), nitrogen dioxide (2.5 ppm), sulfur dioxide (5 ppm) and particles. The particulate phase consisted of either sulfuric acid or ammonium sulfate, with some iron and manganese sulfates. The metallic salts acted as catalysts for the conversion of sulfur [IV] into sulfur [VI], and the adsorption/absorption of gases by the sulfate particles. The investigators noted that nitric acid vapor (HNO_3) was produced within those exposure atmospheres containing both ozone and nitrogen dioxide, and that a significant enhancement of tissue damage occurred with exposure to atmospheres containing sulfuric acid particles or the secondarily produced nitric acid, compared to those containing the less acidic ammonium sulfate. Furthermore, exposure to the stronger acidic atmospheres produced a wider distribution of parenchymal lesions. Thus, the response to the complex atmosphere was different than anticipated based upon the acidity derived solely from the sulfate particles.

In a similar type of study, Bhalla et al. (1987) examined the effects of a complex mixture on epithelial permeability of rat lungs. The exposure atmosphere consisted of ozone (0.6 ppm), nitrogen dioxide (2.5 ppm), sulfur dioxide (5 ppm), ferric oxide (Fe_2O_3; 0.241 mg/m^3), ammonium sulfate (0.308–0.364 mg/m^3), ferric sulfate [$Fe_2(SO_4)_3$; 0.411–0.571 mg/m^3] and manganese sulfate ($MnSO_4$; 0.007–0.009 mg/m^3). As above, nitric acid was found to be produced within the exposure mixture. While epithelial permeability increased immediately following exposure to the mixture and the magnitude of this change was similar to that following exposure to ozone alone, there was an increased persistence of effect after exposure to the mixture, suggesting a role of the secondarily formed acidic species in the biological response.

Interactions may result from the reaction of a gas on the surface of a particle to which it is adsorbed, forming secondary products which may be more toxicologically active than the primary material and which are then carried on the particle to target sites within the respiratory tract. This was suggested to underlie the results of the study of Jakab and Hemenway (1994), cited previously, in which mice were exposed to mixtures of carbon black and ozone. Simultaneous exposure to the

gas and particles produced synergistic interaction, which was suggested to result from the reaction of ozone on the surface of the carbon particles in the presence of adsorbed water, producing surface bound, highly toxic reactive oxygen species.

The secondary production of acidic species as a factor in interaction between gases and particles was examined by Chen et al. (1992), who exposed guinea pigs (1 h) to atmospheres produced by mixing a metal oxide (zinc oxide, ZnO, 0.05 μm) with sulfur dioxide in the presence of water in a high temperature furnace. The metal oxide acted as a catalyst, promoting the oxidation of the sulfur dioxide into sulfuric acid. The result was the production of a coat of sulfuric acid (at concentrations of 0.02 and 0.03 mg/m^3) on the metal oxide particle surface. Animals were also exposed to pure sulfuric acid particles (0.202 mg/m^3) having a similar size as the coated particles. Nonspecific airway hyperresponsiveness was produced in those animals exposed to the acid-coated particles, but not in those exposed to the metal oxide alone. In addition, a similar quantitative change was noted in the animals exposed to the pure acid droplet even though the exposure concentration was about tenfold that of the acid present as a surface coat. The authors' suggested that exposure to particles under conditions which promote the formation of acid as a surface coating can induce adverse effects at low acid concentrations. However, a complication in this study is that the number of particles in the acid-coated atmosphere was greater than in the pure acid atmosphere, and this may also have contributed to the greater potency of the former, as discussed below.

Chemical reactions may occur not only within the exposure atmosphere but within the lungs following deposition of mixture components. Last and Warren (1987) exposed rats to nitrogen dioxide (5 ppm) alone, or in combination with sodium chloride (NaCl) particles (1 mg/m^3). A synergistic interaction was found when collagen synthesis rate of lung minces or protein content of lavage fluid was measured. The investigators suggested that the interaction resulted from the formation of acidic species, e.g. hydrochloric acid (HCl), nitrous acid (HNO_2), or nitric acid, from nitrosyl chloride (NOCl) following its hydrolysis after deposition in the lungs. The nitrosyl chloride was suggested to be produced from a chemical reaction between nitrogen dioxide and sodium chloride within the exposure atmosphere.

4.3. Alteration of the pulmonary environment

An interaction may occur when a component of a mixture potentiates the response to other constituents by producing local changes within the lungs that enhance the toxic action of one or more of the co-inhalants. This may involve a component which itself has toxic potential. Last (1989) hypothesized that synergism between oxidant gases with acidic sulfate particles would result from a shift in the local microenvironmental pH of the lung following deposition of the acidic particles, enhancing the effects of the co-inhaled gas by producing a change in the reactivity or residence time of reactants, such as free radicals, involved in oxidant-induced tissue injury.

This hypothesis was examined in rats exposed to various aerosols (sulfuric acid, ammonium sulfate, sodium sulfate, sodium chloride) with and without oxidant gases (ozone or nitrogen dioxide). Acidic sulfate aerosols alone did not produce any response at concentrations that resulted in a synergism in conjunction with the gases. Evidence that the synergism was due to particle acidity, i.e. hydrogen ion (H^+), was the finding that neither of the nonacidic particles, i.e. sodium sulfate and sodium chloride, was synergistic with ozone, and that there was no difference in response between animals exposed to nitrogen dioxide alone or to nitrogen dioxide in combination with ammonium sulfate, a weakly acidic particle. While ammonium sulfate in combination with ozone, a stronger oxidant than nitrogen dioxide, did result in interaction, significant interaction with the more acidic sulfuric acid occurred at lower ozone concentrations than was noted for mixtures of ozone and ammonium sulfate.

However, local changes in the lung environment may not underlie all interactions between acidic particles and oxidant gases. If the above hypothesis was the only explanation for such interaction, then the effects of ozone should be consistently enhanced by the presence of acid.

This was not observed by Schlesinger et al. (1992a), who exposed rabbits to combinations of ozone at 0.1, 0.3 and 0.6 ppm with sulfuric acid at 0.05, 0.075 and 0.125 mg/m^3 and examined various biological endpoints. Rather than noting synergism in all cases, antagonism was found to occur under some of the exposure conditions. However, interspecies differences in dosimetry and sensitivity of responses cannot be ruled out as contributing to the observed differences between these two studies.

5. Factors modulating interaction

Gas/particle interactions are influenced by environmental factors, such as exposure concentration, exposure duration and exposure regime, and host factors, such as breathing pattern. The contributions of these are outlined below.

5.1. Particle size and size distribution

The occurrence of an interaction between gases and particles may depend upon the size of the latter. A synergistic response in pulmonary biochemical indices following exposure of rats to mixtures of sulfuric acid (1 mg/m^3) and ozone (0.6 ppm) was noted when the acid particle diameter was 0.5 μm, while no potentiation compared to the ozone-only response occurred when the particle diameter was 0.02 μm (Last et al., 1986). The authors' suggested that similar sites of deposition for ozone and acid particles favored interaction, in that the larger particles which deposited to a greater extent within the bronchioalveolar junction, the major target site for ozone, were most interactive. However, other factors, as yet unknown, may be involved, since the deposition efficiency of the smaller particles should be greater than that of the larger, and the total surface area for the aerosol with the smaller particles is greater than that for the larger as well.

Particle size may also affect interaction due to adsorption. For example, as mentioned previously, for a given mass of particles, the total surface area of an aerosol increases as particle size decreases. Thus, by providing a greater surface area for adsorption, smaller particles may be more effective in eliciting interaction than would larger ones, assuming that broad target regions are similar for both sizes, since specific deposition sites within the respiratory tract may differ. Because all aerosols are characterized not only by some median size but also by a size distribution, the relative amounts of particles of different sizes may also affect interaction with gases.

5.2. Particle number concentration

It is sometimes difficult to separate the effects of particle size from particle number since, to obtain the same mass concentration, the number of particles in an aerosol increases as the size of the constituent particles decreases. Various biological responses have been found to occur in guinea pigs following exposure to ultrafine (< 0.1 μm) metal oxide particles (ZnO) having a surface layer of sulfuric acid, and these responses were much greater than were found following exposure to sulfuric acid in pure droplet form, yet having a similar size and mass concentration of sulfate (Amdur and Chen, 1989; Chen et al., 1991).

One explanation for the above results, as noted previously, is that acid is inherently more toxic when adsorbed onto a solid particle than when deposited as an aqueous droplet. On the other hand, another potential factor underlying these results is particle number concentration within the exposure atmospheres. Because of the manner in which the coated particles were produced, at an equal total sulfate mass concentration, sulfuric acid would exist on many more particles when layered on carrier particles than when dissolved into aqueous droplets. Therefore, it is possible that the greater the number of particles containing sulfuric acid, the greater will be the number of respiratory tract target sites affected following deposition, and the more severe will be the overall biological response. This could account for the finding that much higher mass concentrations of pure acid particles were needed to produce the same effect compared to when the acid was present as a surface coat on a solid particle. A recent study (Chen et al., 1995) has indeed confirmed that the number of particles in the exposure atmosphere, not just total mass concentration of hydrogen ion, is an important factor in the production of lung injury following acid sulfate in-

halation, and that there is apparently a threshold for both number and mass concentration for particles to produce a biological response.

While the number concentration of particles within an aerosol may determine the occurrence of a gas/particle interaction, it is sometimes quite difficult to separate this factor from others. Chen et al (1991) exposed guinea pigs to pure sulfuric or to acid coated on ultrafine metal oxide particles. Exposure to ozone (1 h) following exposure to aqueous acid particles (0.3 mg/m^3; 0.09 μm) did not alter the response due to the acid alone. However, when single (1 h) or multiple (3 h/day, 7 days) exposures to the acid-coated particles (0.024 or 0.084 mg/m^3 equivalent H_2SO_4) was followed by exposure to ozone (0.15 ppm), the effect was greater than additive, suggesting synergism. While this infers that the manner in which the acid is delivered, i.e. as a surface coat or as a pure acid particle, affects whether or not any interaction occurs, it is also possible that the presence or absence of interaction also reflects differences in the number concentration of particles in the two types of acidic aerosols.

5.3. Exposure sequence

Although most interaction studies involved simultaneous exposure to all constituents of a mixture, exposure to one agent may alter the response to others which are subsequently inhaled. Thus, the order of exposure may be significant in eliciting a toxic interaction. Interactions involving sequential exposures are important, since there is great potential for such scenarios under ambient conditions.

Gardner et al. (1977) found an additive increase in microbial infectivity when mice were exposed to ozone (0.1 ppm) for 3 h immediately before exposure to sulfuric acid (0.9 mg/m^3, 0.2 μm) for 2 h, while no difference at all from control was found when the acid particles were administered prior to ozone. Grose et al. (1980) exposed hamsters to ozone (0.1 ppm) for 3 h followed by sulfuric acid (1.09 mg/m^3, 0.3 μm) for 2 h. A reduction in ciliary activity in isolated tracheal cultures was observed, and the magnitude of the change was significantly less than that found with exposure to the acid alone, while ozone alone produced no change at all. In this case, preexposure to the ozone reduced the effect of the acid. Finally, Chen et al. (1991), as discussed above, examined the reverse exposure scenario, namely, sulfuric acid followed by ozone, and suggested that prior exposure to acid increased the 'susceptibility' to subsequent exposure to ozone.

The order of exposure also influenced the results of another study (Jakab and Hemenway, 1994), in which mice were exposed for 4 h to carbon black (10 mg/m^3; 2.4 μm) either prior to or after exposure to ozone (1.5 ppm), and then to both materials simultaneously. Simultaneous exposure resulted in synergism, while exposure to carbon either before or after ozone produced responses which were the same as those due to ozone alone. Interaction following simultaneous inhalation of the gas and particle was suggested to be the result of reaction of ozone on the surface of the carbon particles in the presence of adsorbed water, producing surface bound, highly toxicologically active reactive oxygen species. Production of these oxygen species would not occur when the exposures were performed sequentially, accounting for the lack of interaction with this exposure scenario.

5.4. Exposure duration

The occurrence of an interaction is often a function of the duration of exposure. For example, Warren et al. (1986) found synergistic interaction following 7 days of exposures of rats to mixtures of ozone (0.2 ppm) and ammonium sulfate (5 mg/m^3). However, exposures for only 3 days produced responses which were not different from those noted with ozone alone. On the other hand, McGovern et al. (1995) found that exposures of rabbits for 5 days (3 h/day) to mixtures of ozone and sulfuric acid altered pharmacological function of macrophages either synergistically or antagonistically, while exposures for 20 days resulted in no evidence for any interaction. Thus, increasing the duration of exposure may result in interaction reflecting cumulative dose needed for such interaction, or may result in adaptation to one or more of the mixture components, quenching any interaction.

Exposure duration may affect the type of interaction which occurs. For example, Schlesinger et al. (1992b) exposed rabbits (2 h/day, 5 days/week) to a mixture of ozone (0.1 ppm) and sulfuric acid (0.125 mg/m^3), and noted a synergistic increase in bronchial epithelial cell numbers after 4 months of exposure, but antagonism following 8 months of exposure.

5.5. Exposure concentration

Exposure concentration is clearly related to response when pollutants are inhaled singly. While the concentrations of each constituent within a mixture also influence the type or occurrence of any interaction, the actual dose-response curve for a particular toxicant may differ when inhaled alone compared to when inhaled as a constituent of a mixture. Last (1989) observed an apparent 'all-or-none' response in rats exposed to acid sulfate/ozone mixtures, in that there was no dose-response relationship between the concentration of acid in the mixture and the extent of change in various endpoints compared to effects observed with ozone alone. Similarly, Schlesinger et al. (1992a) noted that the concentration of ozone in mixtures of ozone and sulfuric acid to which rabbits were exposed did not necessarily affect the response compared to that found with acid alone.

5.6. Biological endpoint

The observation of an interaction following exposure to a mixture depends upon the specific biological endpoint assessed. This was clearly shown by Schlesinger et al. (1992a), in which identical exposures to mixtures of sulfuric acid and ozone resulted in either no interaction, synergism or antagonism, depending upon the specific endpoint examined.

5.7. Host factors

While interaction is clearly modulated by environmental exposure factors, host factors also play a role. For example, breathing pattern during exposure can affect the response to a mixture. Thus, exercise seemed to potentiate nasal and parenchymal lesions following exposure of rats to a complex mixture containing strong acids, compared to effects with exposure at rest (Kleinman et al., 1989). Interaction, which was manifested both by synergism occurring at lower exposure concentrations of the constituent pollutants than was found to occur with exposure at rest and by an increase in the response at similar exposure concentrations compared to rest, was ascribed to an increase in the delivered dose or dose rate due to a greater minute ventilation with exercise. Changes in breathing pattern may also result from the inhalation of a sensory irritant component of a mixture, and can also result in alteration of the deposition pattern of inhaled toxicants.

Route of exposure is another factor likely modulating interaction. For example, if inhaled through the mouth, a greater amount of a material may reach the lungs than if inhaled through the nose, and changes in breathing from nasal to oronasal may, therefore, affect the potential for interaction when mixtures are inhaled.

6. Conclusion

The occurrence and nature of any interaction between gases and particles is dependent upon numerous factors, including the biological endpoint examined and the specific conditions of each study, such as exposure method, dose and the physicochemical characteristics of the exposure atmosphere (Calabrese and Yang, 1988; NRC, 1988; Kleinman et al, 1989). Because of this dependence upon exposure conditions, it is often very hard to relate different studies performed under different conditions of toxicant concentration and exposure duration. But even within one study examining one particular endpoint, the type of interactions observed may vary depending upon the exposure dose (Calabrese and Yang, 1988). Thus, any description of interactions is really valid only for the specific conditions of the study in question, and cannot be generalized to all conditions of exposure to a particular chemical mix. The inability to predict a priori from the presence of certain pollutants in a complex exposure atmosphere exactly whether there will be an interaction and, if so, what type it will be, complicates attempts to relate laboratory studies of mixtures to actual ambient exposures.

One of the inherent problems in toxicological studies involves the need to extrapolate to humans. This adds an additional complication in the attempt to determine health risks from exposure to pollutant mixtures. For example, there is often much uncertainty as to whether effects at high concentrations will occur at lower pollutant levels normally found in ambient air. The inability to address this issue is often due to a lack of knowledge of the exposure concentration-response relationships for many agents when present as constituents within a mixture, but in many cases there is also lack of knowledge of the individual chemical dose-response functions as well. Furthermore, interaction studies have not as yet addressed the issue of susceptible populations who may be at increased risk from interactions occurring with low concentrations of gases and particles, levels to which normal individuals may not respond. All of these factors make it quite difficult to establish the risk of multichemical exposures to all segments of the population.

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