Environmental Health Perspectives
Vol. 47, pp. 283-292, 1983

Role of Inhalation Studies with Animals in Defining Human Health Risks for Vehicle and Power Plant Emissions

by Roger O. McClellan*

Automotive vehicles and power plants using fossil fuels emit a complex array of gases and particulate material. The physical and chemical characteristics of these emissions vary markedly between sources and comprise only a portion of the contributors to air pollution exposure of people. Further, it is well recognized that a single form of self-inflicted air pollution, cigarette smoking, is the dominant cause of air pollution-induced disease. These factors minimize our potential for developing an adequate understanding of the health effects of vehicle and power plant emissions by studying only people. The alternative is to use the human data to the extent feasible and complement it with information gained in studies with macromolecules, organelles, cells, tissues and whole animals. Within this context, this paper reviews the use of inhalation studies with animals for defining human health risks of airborne materials, especially particulate materials. The major areas covered are: the fate of inhaled materials, the pathogenesis of disease induced by inhaled materials and long-term animal studies to identify late-occurring effects. Emphasis is placed on the utility of studies in whole animals as integrative models in which the multiple processes such as xenobiotic metabolism, cell injury, repair, transformation and promotion under the influence of many host factors interact in a manner that may not be directly observed in isolated cells or tissues.

Introduction

It is well recognized that automotive vehicles and power plants using fossil fuels release large quantities of gases and particles to the atmosphere. These are the products of complete and incomplete combustion as well as entrained noncombustible materials. If the fuels used were totally combustible and combustion were perfect, the emissions would be simple in character. Unfortunately, these conditions are not met completely, and a small portion of the original fuel is released as a complex mixture of gases and particles. The physical and chemical characteristics of the emissions are quite variable because of variations in fuel composition, temperature and time profiles for combustion and postcombustion treatment. There is no typical combustion process emission. This poses a difficult problem for the epidemiologist or experimentalist who is interested in defining the health risks of combustion process emissions. It is obvious that it is difficult, if not impossible, to find a human population that has been exposed to the products of a single combustion process. On the other hand, laboratory animals exposed to emissions from a single combustion process are not typical of the "real world." Further, information gained in animals may only have a limited degree of applicability to understanding the effects of emissions from other combustion processes or these emissions in combination with other materials in the environment.

The heterogeneity of the exposure is matched by the heterogeneity and the nonspecific nature of the biological responses resulting from exposure to

*Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, P.O. Box 5890, Albuquerque, NM 87185.

combustion products. A wide range of functional diseases (with associated structural changes) as well as neoplasms have been identified as being caused by, or at a minimum associated with, exposure to compounds in combustion process emissions. Also, it is unlikely the occurrence of any of these diseases is exclusively related to such exposures. From the foregoing, it should be obvious that the problem of assessing the potential health risks of exposure to combustion process emissions is difficult and certainly more so than defining the risks of exposure to a simple chemical compound.

Faced with these complexities, how can we define the health risks of human exposure? As a starting point, we can glean as much information as possible by conducting epidemiological studies. The chief result has been to establish the association between self-inflicted exposure to a relatively unique form of pollution, cigarette smoke, and a number of human diseases (*1-4*), the most notable being cancer of the respiratory tract. The overwhelming influence of pollution from this source has made it difficult to determine the potential effects of air pollution from other combustion processes.

However, there are a sufficient number of epidemiological clues as well as the recognition of some general similarities between cigarette smoke and other combustion process emissions that indicate emissions other than cigarette smoke have resulted in disease in the past (*1,3,4*). With the low levels of emissions typical of most industrialized countries today, it is possible that no health effects are being produced by these other emissions, or, if they are being produced, they will be distinguishable from those produced by cigarette smoking only by studying very large populations and discerning subtle differences. Such epidemiological studies may establish useful associations between exposure and disease. However, they are unlikely to provide more than clues as to the mechanisms by which air pollutants cause disease and the role of individual constituents of air pollution.

Having established the boundaries within which epidemiological information bearing on the effects of air pollutants can be obtained, let us consider other complementary types of studies. I emphasize that these are not alternatives, but complementary because whatever other type studies are used, they must be linked back to man. This linkage can only come from the study of people. The complementary types of studies are depicted schematically in Figure 1. To illustrate the point being made, let us briefly consider each type of study.

With regard to macromolecules and organelles, the biotransformation capability of xenobiotic-metabolizing enzymes isolated from tissues and

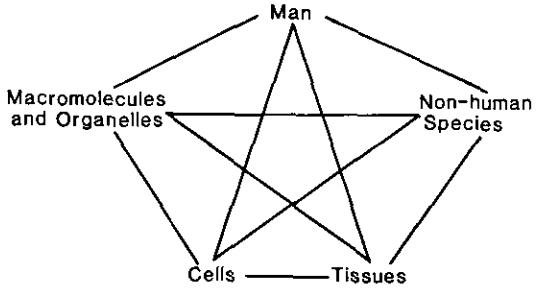

FIGURE 1. Interrelationship between various types of studies used to assess health risks of toxic agents to man.

especially the liver has been recognized for some time. Recently, it has been demonstrated that high concentrations of cytochrome P-450-dependent monooxygenases are present in the nasal epithelium from rats (*5*), dogs (*6*), guinea pigs, rabbits, Syrian hamsters and mice (A. R. Dahl, personal communication). The presence of these enzymes may have great significance in understanding carcinogenesis in the nasal tissues as well as olfactory function. It is assumed that these enzymes are present in human nasal epithelium. However, it is not sufficient to assume their presence; they must be measured in human tissue.

Many assay systems using either bacteria or mammalian cells have become invaluable in demonstrating the cytotoxic, mutagenic and cell transforming activity of environmental pollutants. The reverse mutation assay in bacteria developed by Ames (*7*) represents a classic example of such a test. It provides a quick and relatively inexpensive assay for mutagenic activity and presumptive evidence of carcinogenicity (*8*). However, until the material in question has been demonstrated to be carcinogenic in animals, it cannot be stated that it is a carcinogen. Indeed, some individuals would take the extreme view that until a material has been shown to be carcinogenic in man, it should not be considered a "human carcinogen." Studies with cells isolated from the respiratory tract provide the opportunity to gain insight into the metabolism of compounds of interest in mammalian cells and to observe interactions with the cells' genetic material, thereby building a bridge to the studies in bacteria (*9,10*).

Tissue studies providing linkage between the single cell systems and whole animals are crucial for better understanding the mechanisms of carcinogenesis. The usefulness of tissue systems, for example tracheal explants, for studying effects of pollutants on respiratory tract epithelium have been reviewed (*11*). Because tissues can be readily

obtained both from laboratory animals and man, it is possible to obtain data in several species. Such studies have contributed substantially to our understanding of the metabolism of polynuclear hydrocarbons by the respiratory tract (*12,13*).

Studies in whole animals provide an opportunity to understand how the multiple processes such as cell injury, repair, transformation and promotion interact under the influence of many host factors to yield an observable disease such as lung cancer or emphysema. This is especially critical recognizing the multifactorial nature of the origin of cancer (*14*). In animal studies, the variables can be controlled; this is most frequently not the case for human studies. It is also possible to introduce variables such as toxic agents that would not be appropriate in human studies. It has been possible in some cases to use limited data from man in combination with data from laboratory animal studies to resolve what otherwise appear to be inconsistencies in dose-response data developed in different species including man (*15*).

It is unfortunate that discussions of risk assessment have too often centered on consideration of the utility of a single type of study for predicting health effects in man. To date, the perfect surrogate for man has not been found, and such a surrogate is unlikely to be found in the future. Thus, we are faced with the need to continue our efforts to better understand how to integrate information from several types of studies depicted in Figure 1. In the following sections, I will provide examples of the utility of whole animal studies.

Fate of Inhaled Materials

A major ultimate interest is to obtain reliable estimates for man of the relationship between exposure to pollutants and the subsequent development of an excess of disease. One measure of exposure is the concentration of the pollutant in the medium surrounding man. In the case of vehicle and power plant emissions, air is the medium of particular concern. A distinction must, however, be drawn between exposure and dose. Dose is the actual quantity of material that gains entry to the body or, subsequently, the time course of concentration of the pollutant or specific constituents (or metabolites) within the body, various tissues and, finally, cells and specific targets such as DNA. It is generally accepted that dose, as defined above, is a more appropriate parameter to relate to health effects than is exposure when attempting to establish a relationship that may be used to compare the results of studies with macromolecules, organelles, cells, tissues, laboratory animals and man.

Deposition of Particles

A primary factor determining the dose of an airborne material in man is the fractional deposition of the inhaled material. Fortunately, there is an excellent body of information available on the deposition of particulate materials in man (*16*), and the basic processes (impaction, sedimentation, interception and diffusion) that govern deposition are well understood. The major shortcoming in our knowledge is for ultrafine aerosols (0.5 μm) that have not been studied extensively in man. The deposition of radiolabeled chain aggregates of gallium oxide that resemble diesel soot particles in size and shape have recently been studied in dogs (*17,18*). It was shown that for 0.02 μm and 0.1 μm particles, 32% and 25%, respectively, were deposited in the pulmonary regions of beagle dogs. Approximately one-third as much was deposited in the nasopharyngeal and tracheobronchial regions. The values obtained in the dogs are considered a good estimate for man until such time as ultrafine particle deposition studies can be conducted in people. The validity of the estimate is enhanced by the general agreement previously observed between particle deposition patterns for larger particles in people and beagle dogs (*19*). In addition, the values are only slightly higher than those measured for 0.2 μm particles in man (*20*). This difference may be related to greater diffusional deposition by the particles of 0.02 and 0.1 μm mass median diameter.

Retention of Particles

The data available on clearance, or conversely, retention, of particles in man is more limited than that available on deposition (*16*). Further, most of the particle retention data obtained in people have been for relatively short observation periods. A major deficiency is our lack of knowledge of the long-term respiratory tract retention of particles in man. Some data are currently being obtained in studies being conducted in West Germany and the United Kingdom of persons exposed to radiolabeled micrometer-sized particles.

In the absence of adequate data obtained directly in man, it has been considered appropriate to use studies in laboratory animals to estimate the long-term retention of inhaled materials in man. The validity of the approach has been strengthened by the observation that the long-term retention of zirconium oxide particles labeled with ^{95}Nb in beagle dogs and a man were similar (*21*). In considering the use of data obtained in mice and rats, it must be recognized that they generally clear particles from the lungs more rapidly than do dogs (*22*).

One type of combustion aerosol that is of particular interest is diesel exhaust soot. Chan et al. (*23*) have studied the clearance of radiolabeled diesel exhaust particles in rats and found that a portion of the particles are retained with a clearance half-time of 62 days. These and similar data developed at the Inhalation Toxicology Research Institute (R. Wolff and L. Griffis, personal communication) have been used to predict the accumulated lung burden of diesel soot particles following chronic exposure to diesel exhaust. The predictions have been compared to measured lung burdens of diesel soot particles in rats chronically exposed to diesel exhaust (R. Henderson and W. Hadley, personal communication). The lung burdens of the chronically exposed rats exceed the predicted burdens, especially in the rats at the highest exposure levels where the exposure concentration is on the order of 1 mg/m^3 or higher. The lung retention of the soot particles has also been observed following the cessation of exposure to varying levels of diesel exhaust. A substantial portion of the particles was cleared very slowly with a clearance half-time of over 200 days (*24*). Thus it appears that when large quantities of diesel soot particles are deposited in the lungs of rats, clearance of the particles from the lungs is impaired. There is an indication that the degree of impairment increases with increasing exposure level. If this is the case, then the dose (time-integrated concentration) of particles is not proportional to exposure level; a higher than expected dose occurs at the higher exposure levels. This must be taken into account in extrapolating from the highest exposure levels to ambient levels of exposure relevant to man. Effects observed at these highest levels may be uniquely related to the accumulation of a high burden of diesel soot particles.

A second type of combustion product aerosol of interest is fly ash. Unfortunately, from an experimental viewpoint fly ash is not a single material but will vary in its physical and chemical characteristics dependent upon factors such as the fuel and combustion process (*25,26*). The limited data available on retention of inhaled fly ash in rodents may not adequately predict the retention of all kinds of fly ash. The technique of neutron activation was used in both of the most relevant studies conducted to date. Wehner et al. (*27*) studied fly ash from conventional combustion of coal and observed that ^{46}Sc and ^{59}Fe in the ash served as effective tracers for the matrix of the particles. The longest term component of retention had a biological half-time of just over 30 days. Griffis et al. (*28*) studied fly ash from an experimental fluidized bed combustor. Using ^{46}Sc as the tracer, they observed a long-term component of retention with a biological half-life of 78 days.

Essentially all of the data on retention of the diesel exhaust particles and fly ash have been obtained in rodent species. Recognizing the extent of species differences in long-term clearance, it is important that these rodent studies be duplicated in animals such as dogs or subhuman primates that are generally considered to have a pattern of respiratory tract clearance more like man.

Retention of Trace Elements and Organic Compounds

What was described above is the fate of the matrix of the inhaled particles. The matrix materials, aluminosilicate for the fly ash and carbon for the diesel soot, are generally not considered to be particularly toxic. Attention from a toxicological viewpoint is centered on the trace elements or organic compounds associated with the particles.

The limited studies conducted with fly ash indicate that trace elements such as cobalt are preferentially removed from the fly ash particles in the lung (*27,28*). These materials are available for translocation to other tissues and, thus, concern for potential health effects of the fly ash should not focus exclusively on the respiratory tract.

Interest in the fate of specific chemical constituents associated with particles is increased by recognition that different solvents can remove mutagenic chemicals from both fly ash and diesel exhaust soot particles (*26,29-31*). Some of the mutagenic activity associated with fly ash may be attributed to inorganic compounds. However, at least a part of it is due to organic compounds. In the case of diesel exhaust soot particles, the most mutagenic activity is recovered when the particles are treated with strong organic solvents. This, as well as the identification of specific organic compounds that are known mutagens, focuses attention on the fate of the organic compounds associated with the particles.

Particles contain literally a myriad of individual compounds, with no single compound representing even one percent of the extractable material even when the strongest solvents are used (*32*). The situation becomes even more complex when one considers the release of organic compounds with more biologically relevant extractants *in vivo*. Thus, it has been difficult to identify specific compounds released from diesel exhaust soot under conditions that might be readily extended to the human lung. King et al. (*33*) has reported the release of 1-nitropyrene, a potent bacterial mutagen, in the

presence of lung macrophages. Thus, we have qualitative evidence for the biological availability of specific organic compounds. Since the question of the availability of these compounds for interaction with sensitive biological structures is so critical to bridging the gap between mutagenicity assays in bacteria and effects in animals, it is important to consider alternative approaches.

One such approach has been to study the fate of individual organic compounds instilled or inhaled into the lung. Early work in this area with intratracheally instilled particles was stimulated by the finding that administration of benzo(a)pyrene coated on ferric oxide particles to Syrian hamsters produced a carcinogenic response from the respiratory tract epithelium closely comparable to human bronchogenic carcinoma (*34*). It is now established that the effectiveness of cancer induction in this model is related at least in part to the prolonged retention of the particle-associated benzo(a)pyrene (*35–37*). The influence of particle characteristics on benzo(a)pyrene retention in the lung when intratracheally administered is shown in Figure 2 (*37*). The authors reported that all three types of particles retarded the clearance of benzo(a)pyrene.

In considering the data obtained from intratracheally instilled material, it is appropriate to ask its relevance to inhalation, which represents a more natural mode of entry of material into the lungs. To aid in answering this question, studies have been conducted in rats with benzo(a)pyrene inhaled either coated on particles or as pure benzo(a)pyrene particles (*38*). As with intratracheal instillation, the retention of particle-associated benzo(a)pyrene was prolonged compared to when the pure compound was inhaled (Fig. 2). It should be noted that for both benzo(a)pyrene aerosols, retention was much

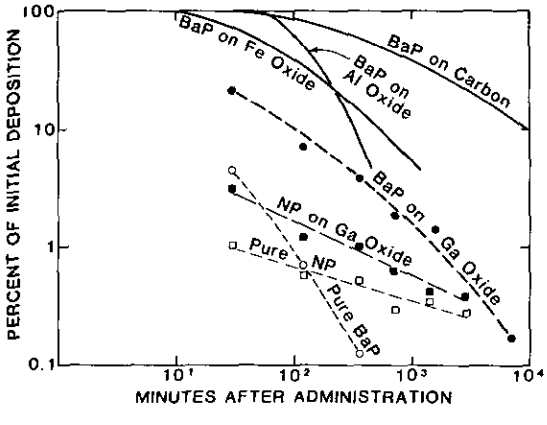

FIGURE 2. Retention in the lung of (—) intratracheally instilled or (---) inhaled benzo(a)pyrene or 1-nitropyrene.

shorter than for the intratracheally instilled forms. Thus, the time integrated concentration of the material in the lung following inhalation is substantially less than for an equivalent amount of intratracheally instilled material. If this parameter is important in determining the effects of the administered material—and I believe it is—this finding may be of substantial toxicological importance. The difference in retention may be related to the more uniform distribution of the inhaled material in the lung and its availability for metabolism. It has previously been shown that inhaled particles are more uniformly distributed in the lung than intratracheally instilled particles (*39*). It is of interest to determine if benzo(a)pyrene associated with diesel soot particles is handled in the same manner. Such studies are now in progress in our Institute.

In view of the interest in nitroaromatic compounds associated with diesel exhaust soot and other fossil fuel combustion products, the fate of a representative compound of this class, 1-nitropyrene, has been evaluated following inhalation. Sun et al. (*40*) exposed rats to this radiolabeled compound by nose-only inhalation exposure either as a coating on gallium oxide particles or as a homogeneous ultrafine aerosol. In contrast to the benzo(a)pyrene results, he found rapid removal of both forms. For the gallium oxide associated form, fecal excretion of the radiolabel predominated (~ 75%). With the pure compound, about three-fourths of the deposited material was excreted in the urine indicating rapid direct absorption into the blood. These results indicate that particle association of the material does modify the fate of inhaled 1-nitropyrene.

With both inhaled benzo(a)pyrene and 1-nitropyrene, there was significant radioactivity translocated to other tissues such as liver and kidney. This emphasizes the need to consider not only respiratory tract effects in evaluating the toxicity of these inhaled aromatic hydrocarbons, but also possible effects in other tissues.

The studies just described utilized radiolabeled (^{3}H) compounds and only the radioactive tracer was followed. Thus, the measurements represent both the parent compound and its metabolites. Although the total portion of the inhaled material that is retained at later time periods is small, it is possible that a substantial portion of it could represent one or more key metabolites. Further work is needed to identify the material that remains in the lung and other tissues, and especially that which may be bound to macromolecules. Also, recognizing potential cellular differences in sensitivity, it would be useful to have more detailed knowledge of the concentration in cells *in vivo* to correlate with data from cultured tissue or cells (*9,10,12,13*).

Health Effects of Inhaled Materials

Effects of Diesel Exhaust

Relatively few long-term studies of the health effects of inhaled vehicle or power plant emissions have been conducted in laboratory animals. Recently, concern over the possible carcinogenicity of diesel exhaust particles has stimulated the conduct of a number of studies. The major studies that have been conducted or are in progress are listed in Table 1. In addition to those listed, other studies will be initiated during the next year in Japan by the Japan Automobile Research Institute, in Switzerland by the Battelle Memorial Institute and in West Germany by the Fraunhofer Institute for Toxicology and Aerosol Science. In considering the studies listed in Table 1, it is important to note that only a few of the studies involve life-span exposure and observation. A special premium should be placed on observation of experimental subjects for their full life-span, recognizing that if cancers are induced they are likely to occur in low incidence and late in life. In addition to the studies shown in Table 1, a number of other studies have been conducted in which animals were exposed to whole exhaust from internal combustion engines and evaluations focused on nonneoplastic end points (*48*).

In studies completed to date, all of the observed health effects of inhalation exposure to automotive emissions have been nonneoplastic in nature. In general, the responses have been similar in all laboratory animals. The responses that are seen appear to relate in some way to the substantial accumulation of diesel exhaust particles in the lungs and tracheobronchial lymph nodes (*44,49–51*).

After inhalation, the biological sequence of events starts with the phagocytosis of particles by alveolar macrophages. With time, there is an increase in both the number and size of macrophages and an increasing concentration of diesel exhaust particles within their cytoplasm. The type II pneumocytes also increase in number and size in the alveoli that contain particle-laden macrophages. Both neutrophils and eosinophils appear to be recruited and to phagocytize particles under conditions of high pulmonary loading. With time, particle-laden macrophages form dense aggregates within alveoli, most notably adjacent to terminal bronchioles. The surrounding tissue response to the macrophage clusters is highly variable. In some instances, there is a proliferation of interstitial cells and an increase in interstitial reticulin but in other cases, there was no elicited response. Particles are also translocated from alveoli to the interstitium where they are usually contained in interstitial macrophages. Finally, it has been shown that particles are transported to local and regional lung-associated lymphoid tissues. Although at later times these tissues concentrate a significant mass of particles within histiocytes, there is no evidence that other surrounding cells are affected by their presence.

The responses in lung and lymph nodes observed to date represent the usual response of lung to inhaled particles of a relatively insoluble form (*51*). Longer-term observations will be required to ascertain whether the lesions remain the same or whether with time, they become more functionally significant. Substantial effort has been directed toward evaluating nonmorphological responses, for example, biochemical and physiological alterations. The biochemical changes observed in tissues and airway fluids have, in general, been transient by nature, suggesting injury followed by adaptation or repair. The physiological changes other than the influence on clearance rates have been minimal to nonexistent even at the highest exposure levels.

Investigators at the Fraunhofer Institute have recognized the difficulty of detecting small carcinogenic effects and have used a novel approach to

Table 1. Major long-term studies of the health effects of diesel exhaust completed or in progress.

Laboratory	Reference	Species	Particle concentration, $\mu g/m^3$	Life-span study	Completed
Environmental Protection Agency	(*41*)	Chinese hamster, mice, rats, cats	6000–12000	No	Yes
Fraunhofer Institute	(*42*)	Syrian hamsters	4200[a]	Yes	Yes
General Motors	(*43*)	Rats, guinea pigs	250, 750, 1500	No	Yes
Lovelace Inhalation Toxicology Research Institute	(*44*)	Mice, rats	350, 3500, 7000	Yes	No
Battelle-Northwest	(*45*)	Rats	8300	Yes	Yes
	(*46*)	Syrian hamsters	7300		
Southwest Research Institute	(*47*)	Syrian hamsters Rats, mice	1:60, 1:120, 1:360 dilution[b]	No	Yes

[a]Also, exposures to gaseous emissions only without particles.
[b]Particle concentrations not given.

attempt to detect such an effect (*42*). They have pretreated Syrian hamsters with subcutaneous injections of either 1.5 or 4.5 mg of diethylnitrosamine per kilogram body weight and then exposed them to diesel engine exhaust. One group received whole exhaust and a second group received exhaust in which the particles had been removed by centrifugation and filtration. The rationale for the study was that the pretreatment would produce an incidence of cancer that would be on the ascending portion of a sigmoid dose-response curve. Thus, a small incremental increase in dose by the exhaust exposure may give rise to a relatively larger increase in the incidence of cancer than if the increase in dose was from zero where the dose-response curve is very flat. No tumors were observed in untreated diesel exhaust-exposed animals. However, the animals pretreated with the highest dose of diethylnitrosamine and exposed to diesel exhaust showed a significantly increased incidence of papillomas of the larynx and trachea compared to the groups receiving only the proven carcinogen (*36*, *52*). There was no statistical difference in the increase in incidence between animals that received whole exhaust or particle-free exhaust. There are several possible interpretations of the data. The enhanced incidence of cancer may be due to carcinogenic activity in the exhaust, the promotional effect of irritant gases that are in the exhaust or to some other factor. The similarity of the response in both groups (with and without particles) suggests the effect may be due to the promotional properties of the irritant gases. It is significant that this is the only inhalation study with diesel exhaust that, to date, has demonstrated an enhanced tumor response.

One study has been conducted in which Strain A mice were exposed to diesel exhaust. This strain has a genetic propensity for developing lung adenomas early in life and at high incidence. Contrary to what might have been expected, the lung tumor incidence was lower in the diesel-exposed mice than in the nonexposed mice (*53*).

Effects with Cigarette Smoke

The negative carcinogenesis findings with diesel exhaust exposure are probably not surprising when considered in light of the results obtained with animals exposed to cigarette smoke. In general, the studies have yielded negative results or only a modest increase in the incidence of respiratory tract cancers (*54–56*). Two studies which yielded positive results are of note. Doutenwill et al. (*55*) conducted chronic exposure studies with Syrian hamsters exposed to cigarette smoke and observed 3610 animals over their life-span. They observed an increased incidence of laryngeal neoplasms that, from the descriptions given, appear to be similar to those observed in the Fraunhofer study with Syrian hamsters exposed to diesel exhaust and diethylnitrosamine. It is of interest that Doutenwill et al. (*55*) did not report an enhanced effect in animals exposed to cigarette smoke for one year and then treated with diethylnitrosamine. Because of the timing of the administration of the nitrosamine and the cigarette exposure, this was not an adequate test of the promotional properties of the cigarette smoke. Thus, it is not strictly comparable to the Fraunhofer study with diesel exhaust exposure after nitrosamine treatment.

The study by Dalbey et al. (*56*) of cigarette smoke exposure of Fischer 344 rats is of interest for two reasons. First, the authors view it as the only study in which an unequivocal tumor response in the respiratory tract resulted from long-term cigarette smoke exposure. It is noteworthy that the difference in incidence in the exposed (9%) versus controls (1%) was modest despite the lifetime exposure. Second, the strain of rats used was the same as that being used in the Inhalation Toxicology Research Institute study (*44*) of the effects of lifetime exposure of rats and mice to diesel exhaust. Ultimately, a comparison of the results of the two studies should provide an indication of the relative effectiveness of exposure to high levels of diesel exhaust versus cigarette smoke.

Effects with Benzo(a)pyrene

Of the various constituents of combustion emissions, benzo(a)pyrene has been the most extensively studied for its carcinogenic activity. Two studies have yielded positive results. Thyssen et al. (*57*) exposed Syrian hamsters to 2.2, 9.5 or 45.6 mg of benzo(a)pyrene/m^3 for 3 to 4.5 hr/day, 7 days per week. This resulted in a "dose" of 29, 127 or 383 mg of benzo(a)pyrene to the three groups. At the highest level, severe toxic effects were noted as well as neoplasms. The highest incidence of neoplasms found in the trachea and larynx was found in the intermediate dose group (over 50%). Bronchiogenic tumors or tumors of the lungs were not observed.

The second positive study is of particular interest since it involved exposure of rats and Syrian hamsters to benzo(a)pyrene and the irritant, sulfur dioxide (*58*, *59*). An increased incidence of squamous cell carcinomas were observed in the rats with a combined treatment of benzo(a)pyrene plus SO_2. An increased incidence was not seen in animals receiving either treatment alone nor in Syrian hamsters. One of the investigators (*59*), commenting on the rat study noted, "one might be tempted to

overinterpret these findings as a demonstration of the hazardous nature of town air, but I would caution that it might be more realistic to suggest that we have simulated cigarette smoking where exposure to polycyclic hydrocarbon carcinogens is combined with exposure to irritant substances such as oxides of nitrogen, phenols, aldehydes, etc."

Taken together, the results of the few positive studies lend support to the multifactorial etiology of cancer of the respiratory tract. They suggest the utility of conducting future studies to further assess both the initiating and promotional properties of combustion product emissions.

Statistical Considerations

The foregoing studies need to be considered in light of the major statistical problem that is faced in all animal studies, and indeed also in studies with other systems. It is the problem of detecting low frequency, but high consequence effects. The issue is apparent when one considers the level of risk that is of concern for human populations; a risk on the order of one excess case of a disease per year in a population of ten thousand might be viewed as excessive. For a population of 200 million people, this would represent 20,000 excess cases per year. For reference purposes, this represents a risk equal to one-fourth of the lung cancer risk attributed to cigarette smoking in the United States. Deaths from lung cancer attributed to smoking in the United States account for about 4% of all fatalities annually. Although cigarette smoking is tolerated as a social habit, it is clear that one would not want to expose large populations to other equivalent risks.

It is immediately obvious that a risk of this magnitude will not be detected even by studying tens of thousands of animals exposed at levels that are similar to those of concern for man. The only choice, if animal studies are to be conducted, is to utilize levels of exposure and associated doses that are substantially greater than those likely to be encountered by people. The extent of the problem is apparent from consideration of the statistical relationship shown in Figure 3. The size of the animal population required for detection of a statistically significant increase in the incidence of an effect above that in a control population can be readily calculated using standard methods (*60*). In the example shown, 50 animals would allow detection of a 20% excess incidence at 95% statistical confidence, assuming a control incidence of 1%. However, 130 animals would be needed to detect a 10% excess incidence or 400 animals to detect a 5% excess incidence with the same level of confidence. Two things are apparent from the foregoing. First, the level of excess risk detectable in a reasonable-sized animal population is of the same order of magnitude as that associated with the most significant source of air pollution to man—cigarette smoking. However, the total dose of cigarette smoke received by people is probably more than 20 times that received by rodents on a similar smoking schedule due to their differences in life spans. Second, although the exposure duration and dose relationships could be solved by using much larger populations, these attempts are soon defeated by the diminishing returns of larger populations, e.g., the increase in sensitivity is not proportional to population size above 100 animals or so.

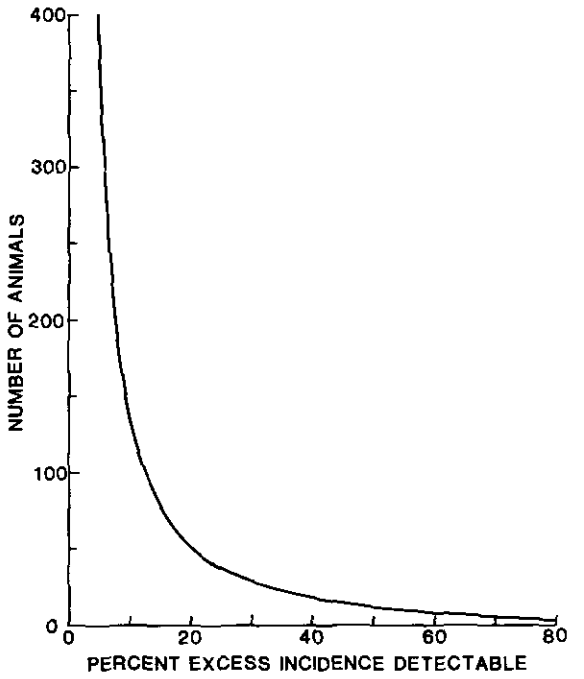

FIGURE 3. Relationship between the number of animals in an experimental population and the detectable incidence of an effect in excess of the incidence in a control population. The example shown was calculated by using a published equation (*61*), assuming 95% statistical confidence and power and a 1% incidence in the control population.

With the statistical issue in mind, the results of the animal studies that have been conducted with vehicle emissions cannot be viewed as proving the lack of carcinogenicity of vehicle exhaust. At best, they lend support to the notion that the use of an increased number of diesel vehicles is unlikely to result in an epidemic of lung cancer. On the other hand, consideration of the negative or weakly positive results with animals exposed to cigarette smoke suggests that the results obtained to date with diesel exhaust exposure should be interpreted with caution.

I acknowledge with gratitude the numerous valuable discussions I have had with my colleagues concerning the topics covered in this paper as well as their role in conducting many aspects of the work I have reported. I especially note the assistance of Dr. J. D. Sun for preparing Figure 2 and Dr. J. L. Mauderly for preparing Figure 3.

This report was prepared under U. S. Department of Energy Contract Number DE-AC04-76EV01013.

REFERENCES

1. Doll, R., and Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J. Natl. Cancer Inst. 66: 1191-1308 (1981).
2. U.S. Department of Health Education and Welfare. Smoking and health: a report of the Surgeon General. DHEW Publication No. (PHS) 79-50066, U.S. Government Printing Office, Washington, DC, 1979.
3. Friberg, L., and Cederlof, R. Late effects of air pollution with special reference to lung cancer. Environ. Health Perspect. 22: 45-66 (1978).
4. Karch, N. J., and Schneiderman, M. A. Explaining the urban factor in lung cancer mortality. Clement Associates Inc., Washington DC, 1981.
5. Hadley, W. M., and Dahl, A. R. Cytochrome P-450 dependent monooxygenase activity in rat nasal epithelial membranes. Toxicol. Letters 10: 417-422 (1982).
6. Dahl, A. R., Hadley, W. M., Hahn, F. F., Benson, J. M., and McClellan, R. O. Cytochrome P-450-dependent monooxygenases in olfactory epithelium of dogs: possible role in tumorigenicity. Science 216: 57-58 (1982).
7. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens as mutagens with the *Salmonella*/mammalian microsome mutagenicity test. Mutat. Res. 31: 347-364 (1975).
8. Ames, B. N. Identifying environmental chemicals causing mutagens and cancers. Science 204: 587-593 (1979).
9. Feldman, G., Remsen, J., Wang, T. V., and Cerutti, P. Formation and excision of covalent deoxyribonucleic acid adducts of benzo(a)pyrene 4,5-epoxide and benzo(a)pyrenediol epoxide I in human lung cells. Biochemistry 19: 1095-1101 (1980).
10. Teel, R. W. Induction of aryl hydrocarbon hydroxylase in primary cultures of Type II alveolar lung cells and binding of metabolically activated benzo(a)pyrene to nuclear macromolecules. Cancer Letters 7: 349-355 (1979).
11. Nettesheim, P., and Griesemer, R. A. Experimental models for studies of respiratory tract carcinogenesis. In: Pathogenesis and Therapy of Lung Cancer, C. C. Harris (ed.), Marcel Dekker, New York, 1978, pp. 75-188.
12. Autrup, H., Harris, C. C., Stoner, G. D., Selkirk, J. K., Schafer, P. W., and Trump, B. F. Metabolism of [^{3}H]benzo-(a)pyrene by cultured human bronchus and human pulmonary alveolar macrophages. Lab. Invest. 38: 217-224 (1978).
13. Cohen, G. M., Marchok, A. C., Nettesheim, P., Steele, V. E., Nelson, F., Huang, S., and Selkirk, J. K. Comparative metabolism of benzo(a)pyrene in organ and cell cultures derived from rat tracheas. Cancer Res. 39: 1980-1984 (1979).
14. Saffiotti, U. Occupational carcinogenesis in relation to the multifactorial origin of cancer: experimental pathology approaches. In: The Prevention of Occupational Cancer (International Symposium, Occupational Safety and Health Series, No. 46), International Labour Office, Geneva, 1982, pp. 17-25.
15. Gehring, P. J., Watanabe, P. G., and Park, C. N. Resolution of dose-response toxicity data for chemicals requiring metabolic activation: example—vinyl chloride. Toxicol. Appl. Pharmacol. 44: 581-591 (1978).
16. Lippmann, M., Yeates, D. B., and Albert, R. E. Deposition, retention and clearance of inhaled particles. Brit. J. Ind. Med. 37: 337-362 (1980).
17. Kanapilly, G. M., Wolff, R. K., DeNee, P. B., and McClellan, R. O. Generation, characterization and deposition of inhaled ultrafine aggregate aerosols. In: Proceedings of BOHS 5th International Symposium on Inhaled Particles, Cardiff, England, September 8-12, 1980, in press.
18. Wolff, R. K., Kanapilly, G. M., DeNee, P. B., and McClellan, R. O. Deposition of 0.1 μm chain aggregate aerosols in Beagle dogs. J. Aerosol Sci. 12: 119-129 (1981).
19. Cuddihy, R. G., Brownstein, D. G., Raabe, O. G., and Kanapilly, G. M. Respiratory tract deposition of inhaled polydisperse aerosols in beagle dogs. J. Aerosol Sci. 4: 35-45 (1973).
20. Chan, T. L., and Lippmann, M. Experimental measurements and empirical modelling of the regional deposition of inhaled particles in humans. Am. Ind. Hyg. Assoc. J. 41: 399-409 (1980).
21. Waligora, S. J., Jr. Pulmonary retention of zirconium oxide (^{95}Nb) in man and beagle dogs. Health Phys. 20: 89-91 (1971).
22. Thomas, R. G. An interspecies model for retention of inhaled particles. In: Assessment of Airborne Particles, Charles C Thomas, Rochester, NY, 1972, pp. 405-420.
23. Chan, T. L., Lee, P. S., and Hering, W. E. Deposition and clearance of inhaled diesel particles in the respiratory tract of Fischer rats. J. Appl. Toxicol. 1: 77-82 (1981).
24. Griffis, L. C., Wolff, R. K., Henderson, R. F., Griffith, W. C., Mokler, B. V. and McClellan, R. O. Clearance of diesel soot particles from rat lung after a subchronic diesel exhaust exposure. Submitted for publication.
25. Carpenter, R. L., Newton, G. J., Rothenberg, S. J., and DeNee, P. B. Respirable aerosols from fluidized bed coal combustion II. Physical characteristics of fly ash. Environ. Sci. Technol. J. 14: 853-859 (1980).
26. Fisher, G. L. Biomedically relevant chemical and physical properties of coal combustion products. Environ. Health Perspect. 47: 189-200 (1983).
27. Wehner, A. P., Wilkerson, C. L., Mahaffey, J. A., and Milliman, E. M. Fate of inhaled fly ash in hamsters. Environ. Res. 22: 485-498 (1980).
28. Griffis, L. C., Snipes, M. B., and Brooks, A. L. Clearance by the rat of inhaled fly ash from fluidized bed coal combustion. J. Toxicol. Environ. Health 7: 117-124 (1981).
29. Lewtas, J. Evaluation of the mutagenicity and potential carcinogenicity of combustion emissions using short-term bioassays. Environ. Health Perspect. 47: 141-152 (1983).
30. Vostal, J. J. Bioavailability and biotransformation of the mutagenic component of particulate emissions present in motor exhaust samples. Environ. Health Perspect. 47: 269-282 (1983).
31. Clark, C. R., and Hobbs, C. H. Mutagenicity of effluents from an experimental fluidized bed coal combustor. Environ. Mutagen. 2: 101-109 (1980).
32. Schuetzle, D. Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspect. 47: 65-80 (1983).
33. King, L. C., Loud, K., Tejada, F. B., Kohan, M. J., and Lewtas, J. L. Evaluation of the release of mutagens and 1-nitropyrene from diesel particles in the presence of lung macrophages in culture. Submitted for publication.
34. Saffiotti, U., Cefis, F., and Kolb, L. H. A method for the experimental induction of bronchiogenic carcinoma. Cancer Res. 28: 104-124 (1968).
35. Henry, M. C., Port, C. D., and Kaufman, D. G. Importance of physical properties of benzo(a)pyrene-ferric oxide mixtures in lung tumor induction. Cancer Res. 35: 207-217 (1975).

36. Saffiotti, U. Evaluation of mixed exposures to carcinogens and correlations of *in vivo* and *in vitro* systems. Environ. Health Perspect. 47: 319-324 (1983).
37. Henry, M. C., and Kaufman, D. G. Clearance of benzo(a)pyrene from hamster lungs after administration on coated particles. J. Natl. Cancer Inst. 51: 1961-1964 (1973).
38. Sun, J. D., Wolff, R. K., and Kanapilly, G. M. Deposition, retention and biological fate of inhaled benzo(a)pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol. Appl. Pharmacol., submitted.
39. Brain, J. D., Knudson, D. E., Sorokin, S. P., and Davis, M. A. Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ. Res. 11: 13-33 (1976).
40. Sun, J. D., Wolff, R. K., and Aberman, H. M. Inhalation of 1-nitropyrene associated with ultrafine insoluble particles or as a pure aerosol: a comparison of deposition and biological fate. Submitted for publication.
41. Pepelko, W. E. EPA studies on the toxicological effects of inhaled diesel engine emissions. In: Toxicological Effects of Emissions from Diesel Engines, J. Lewtas (Ed.), Elsevier-North Holland, Amsterdam, 1982.
42. Heinrich, U., Peters, L., Funcke, W., Pott, F., Mohr, U., and Stober, W. Investigation of toxic and carcinogenic effects of diesel exhaust in long-term inhalation exposure of rodents. In: Toxicological Effects of Emissions from Diesel Engines, J. Lewtas (Ed.), Elsevier-North Holland, Amsterdam, 1982.
43. Schreck, R. M., Soderholm, S. C., Chan, T. L., Hering, W. E., D'Arcy, J. B., and Smiler, K. L. In: Health Effects of Diesel Engine Emissions, Vol. 2, W. E. Pepelko, R. M. Danner, and N. A. Clarke (Eds.), U.S. EPA, Cincinnati, 1980, p. 573.
44. Mauderly, J. L., Benson, J. M., Bice, D. E., Henderson, R. F., Jones, R. K., McClellan, R. O., Mokler, B. V., Pickrell, J. A., Redman, H. C., and Wolff, R. K. Life span study of rodents inhaling diesel exhaust: Experimental design and results through six months. In: Inhalation Toxicology Research Institute Annual Report 1980-1981, LMF-91, pp. 312-330, DOE Research and Development Report, December 1981.
45. Cross, F. T., Palmer, R. K., Filipy, R. E., Busch, R. H., and Stuart, B. O. Study of the combined effects of smoking and inhalation of uranium ore dust, radon daughters and diesel oil exhaust fumes in hamsters and dogs. Pacific Northwest Laboratory Report 2744.
46. Karagianes, M. T., Palmer, R. F., and Busch, R. H. Effect of inhaled diesel emissions and coal dust in rats. Am. Ind. Hyg. Assoc. J. 42: 382-391 (1981).
47. Springer, K. J. SWRI-SFRE diesel health effects exposure facility. In: Toxicological Effects of Emissions from Diesel Engines, J. Lewtas (Ed.), Elsevier-North Holland, Amsterdam, 1982.
48. Ackermann, W., Funcke, W., Heinrich, U., Konig, J., Muhle, H., Oberdorster, G., Peters, L., Pott, F., and Stober, W. Literature study of animal experiments and epidemiological studies of the biomedical effects of exhaust from internal combustion engines (gasoline and diesel engines). Report prepared by Fraunhofer Institute of Toxicology and Aerosol Research, Munster, West Germany, 1980.
49. Pepelko, W. E., Danner, R. M., and Clarke, N. A. (Eds.). Health Effects of Diesel Engine Emissions, Vols. 1 and 2. U. S. Environmental Protection Agency, Cincinnati, 1980.
50. Lewtas, J. (Ed.). Toxicological Effects of Emissions from Diesel Engines. Elsevier-North Holland, Amsterdam, 1982.
51. McClellan, R. O., Brooks, A. L., Cuddihy, R. G., Jones, R. K., Mauderly, J. L., and Wolff, R. K. Inhalation toxicology of diesel exhaust particles. In: Toxicological Effects of Emissions from Diesel Engines, J. Lewtas (Ed.), Elsevier-North Holland, Amsterdam, 1982.
52. Pott, F., and Stober, W. On the carcinogenicity of airborne combustion products observed in subcutaneous tissue and lungs of laboratory rodents. Environ. Health Perspect. 47: 293-303 (1983).
53. Pepelko, W. E., Orthoefer, J. G., and Peirano, W. B. The influence of inhaled diesel engine emissions upon lung tumor induction in Strain "A" mice. Paper presented at U. S. Environmental Protection Agency 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
54. Binns, R. Animal inhalation studies with tobacco smoke (a review). Rev. Environ. Health 2: 81-116 (1975).
55. Dontenwill, W., Chevalier, H. J., Harke, H. P., Lafrenz, U., Reckzeh, G., and Schneider, B. Investigations on the effects of chronic cigarette-smoke inhalation in Syrian Golden hamsters. J. Natl. Cancer Inst. 51: 1781-1832 (1973).
56. Dalbey, W. E., Nettesheim, P., Griesemer, R., Caton, J. E., and Guerin, M. R. Chronic inhalation of cigarette smoke by Fischer-344 rats. J. Natl. Cancer Inst. 64: 383-390 (1980).
57. Thyssen, J., Althoff, J., Kimmerie, G., and Mohr, U. Inhalation studies with benzo(a)pyrene in Syrian Golden hamsters. J. Natl. Cancer Inst. 66: 575-577 (1981).
58. Laskin, S., Kuschner, S., and Drew, R. T. Studies in pulmonary carcinogenesis. In: Inhalation Carcinogenesis. M. G. Hanna, P. Nettesheim and J. R. Gilbert (Eds.), U. S. Atomic Energy Commission, Division of Technical Information, Oak Ridge, TN, 1970, p. 321.
59. Kuschner, M. The causes of lung cancer. Am. Rev. Respir. Dis. 98: 573-590 (1968).
60. Snedecor, G. W., and Cochran, W. G. Statistical Methods, 6th ed. Iowa State University Press, Ames, Iowa, 1967, pp. 221-223.