Consensus Report: Mutagenicity and Carcinogenicity of Car Exhausts and Coal Combustion Emissions*

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Introduction

Car exhausts and coal combustion emissions may cause a spectrum of health effects, varying from annoyance reactions, to bronchitis, to cancer in the respiratory organs and possibly also other organs. Death in cardiovascular diseases in particularly sensitive individuals have furthermore, under certain circumstances, been associated with ambient air pollution. The objective of the meeting was to examine the relevance of short-term and long-term biological tests for mutagenicity and carcinogenicity to the assessment of human carcinogenic risk that may arise from exposure to air pollution from motor vehicle exhausts and coal combustion products. The participants were asked to address some specific questions:

- Is there epidemiological evidence that motor exhausts or emissions from coal-fired power plants and boilers have contributed to the development of human cancer?
- Which mutagenic and carcinogenic substances occur in emissions from different types of motor vehicles and coal combustion plants? How representative are the samples taken at the source of pollution for risk evaluation? Can risk assessments be made on the basis of chemical composition alone of combustion products or ambient air pollution?
- Can whole animal experiments or short-term tests help answering questions regarding the mutagenicity and carcinogenicity of these types of emissions? What prediction can be made about future risks for humans on the basis of animal experiments or short-term tests? Can such tests be valuable for the choice of different types of engines, for the development of emission control technology or for traffic and city planning?

The scientific problems dealt with at this symposium were primarily confined to mutagenic and
cancer risks. The participants were mindful of the fact that biological effects of air pollutants affecting the respiratory system or other organ systems have also to be taken into account when assessing the overall health impact of air pollution.

The summing up of the scientific knowledge in the areas of chemistry, mutagenicity and carcinogenicity studies, and risk assessment and the resulting conclusions and recommendations were made in subgroups at the symposium. These parts of the report were discussed only in the subgroups, and they are thus responsible for this material. All conclusions and recommendations of each subgroup were, however, discussed and finally approved at plenary sessions.

Risk Assessment*

Risk assessment involves two components: the qualitative judgment about the likelihood that the agent under assessment is a carcinogen for humans and the quantitative estimation of the cancer risk at given levels of exposure (1,2).

Potential sources of health effects data for use in risk assessment include epidemiological investigations, whole animal bioassays, and short-term bioassays, with the given order being directly related to human health relevance. The strengths and limitations of each of these approaches have been discussed elsewhere in this document, but are summarized below.

Risk Assessment by Using Epidemiological Data

In the epidemiology section of this report, it is noted that combustion products of fossil fuels in ambient air, probably acting together with cigarette smoke, have been responsible for cases of lung cancer in large urban areas to the extent of 5-10 cases per 100,000 males per year; this corresponds to approximately 10% of lung cancer in large cities or roughly 1-2% of all cancer for the U.S. as a whole. However, epidemiological data have not been sufficient to allow a precise determination of the quantitative association between specific exposure and cancer risk (3,4).

Risk Assessment by Whole Animal Bioassay

Whole animal studies of combustion products or their components have been conducted by inhalation, intratracheal instillation, skin painting and tumor induction by subcutaneous injections. Animal bioassay data and the results of complementary metabolic and mechanistic studies can provide estimates of carcinogenic risk to man. The estimates may be qualitative (nonnumerical weight of evidence), relative (providing linkage to other materials through available human data) or directly quantitative (which must be developed with caution) (1,5,6). So far, confirmed positive results have not been obtained in inhalation or intratracheal instillation studies. On the other hand, there is clear evidence of papilloma and carcinoma formation in the skin painting tests, and both skin and lung tumor formation in the subcutaneous injection test. In addition, tests involving animals initiated with a known carcinogen subsequently exposed to inhalation of complete diesel exhaust or particle-free diesel exhaust, displayed positive results for lung tumors.

Risk Assessment by Short-Term Bioassay

There are situations of practical importance where the use of short-term bioassay methods could be helpful if they accurately measure the comparative carcinogenicity of fossil fuel combustion products: for example, in the evaluation of different type of fuels or different driving cycles in terms of the carcinogenicity of engine exhausts or the effects of various cleaning devices on the carcinogenicity of stack emissions from coal-fired power plants. In this instance, we are not concerned with the absolute numbers of cancers induced in an exposed population by these emissions but rather with the question of the relative carcinogenicity of the different types of emissions.

There is a large and growing number of short-term bioassays for mutagenicity and carcinogenicity (7,8). They mostly measure genotoxicity (DNA damage, mutations and chromosomal abnormalities) or neoplastic cell transformation. By far, the greatest experience has been obtained with bacterial mutagenicity assays where test strains have been developed to enhance the sensitivity and selectivity of response. There is ample evidence to indicate that these bacterial test systems have markedly different sensitivities to different mutagens. Although there is a rough correlation between mutagenicity and carcinogenicity, only a beginning has been made

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systematically to evaluate which of the short-term bioassays, singly or in combination, give the most accurate measure of carcinogenic potency (9,10). Short-term bioassays may be used with caution, preferably in association with parallel evaluations of the chemical characteristics of the materials being examined.

Quantitative Carcinogenic Risk Assessment for Combustion Products of Fossil Fuels

In assessing carcinogenic risks, we need to know not only how likely it is that the material being evaluated is carcinogenic for humans, but also how much cancer the material is likely to cause for a given amount of exposure. We know that fossil fuel combustion products contain carcinogens; the evidence stems from the chemical identification of recognized carcinogens (11–13), positive bioassay data from a variety of in vitro and animal systems (7) as well as epidemiologic studies (3). One of the oldest established occupational carcinogenic hazards, scrotal cancer in chimney sweeps (14), resulted from exposure to fossil fuel combustion products. A central question, therefore, in the assessment of carcinogenic risks from fossil fuel combustion products is how much cancer they are likely to cause. The control of fossil fuel emissions is costly and there is a strong impetus to estimate, even crudely, the magnitude of the public health benefits to be realized from alternative control strategies.

The point of departure for a quantitative risk assessment can be epidemiologic data relating excess cancer to a defined level of carcinogenic exposure, or similar data in animals. At the present time, short-term bioassays alone do not provide an adequate basis for a quantitative assessment of risks.

One possible approach to a quantitative risk assessment, which is being explored in the case of diesel emission particulates, is to determine the comparative potency by a combination of short-term in vitro and whole animal cancer tests of diesel particles in relation to a series of combustion and distillation products (cigarette smoking, coke oven and roof tar fumes) for which there is epidemiologic cancer data. If successful, such epidemiologic data could then serve as the point of departure for a quantitative assessment (15).

When starting with whole animal bioassay data, the quantitative assessment requires a double extrapolation: an estimation of the relative susceptibility of humans and animals and a mathematical basis for extrapolating dose–response relationships from the levels which produce cancer responses in the observed range to those which would be expected to occur from the much lower levels of environmental exposure. In the case of an assessment based on epidemiologic data, only the extrapolation from high to low doses is needed and the uncertainty of extrapolating from animals to man is avoided.

There is no way to determine the actual shape of the dose-response relationship for carcinogens at very low levels of exposure, since the cancer risks of concern in large populations are far below the levels which can be determined directly either by animal or by epidemiologic studies. An extrapolation model cannot be chosen on the basis of the goodness-of-fit in the observable range of cancer response since a wide variety of mathematical models fit equally well in this range and give widely divergent responses at low levels of exposure (16–18).

The linear nonthreshold extrapolation model has had extensive use for quantitative risk estimation beginning in the field of ionizing radiation and extending to chemical carcinogens (1). The biological justification for its use lies in the correlation between carcinogenesis and mutagenesis, commonality of DNA damage for carcinogenic and mutagenic action, the quantal nature of the interactions of chemicals with DNA, and the linear nature of the dose-response relationships for mutagenesis, particularly with organisms such as bacteria, which can be evaluated in large numbers. From a theoretical standpoint, the multistage mathematical model for carcinogenesis has considerable plausibility and a linear component is an integral part of this model. Indeed, a linear dose-response relationship is seen in the initiation stage of the two stage mouse skin tumorigenesis model. From the practical standpoint, the linear nonthreshold extrapolation model has substantial support as one which provides a plausible upper limit for the estimation of carcinogenic risks. It is certainly possible that the linear nonthreshold model may overstate carcinogenic risks for an individual compound or a mixture of carcinogens but, on the other hand, quantitative risk estimates necessarily neglect possible synergistic interactions with other environmental carcinogens.

Quantitative risk estimates, using a linear nonthreshold dose-response extrapolation model, might be found to be useful in the choice of regulatory strategies for fossil fuel combustion products, but such estimate should be regarded as uncertain and should be used with caution.

Conclusions

Some fossil fuel combustion and pyrolysis products contain carcinogens and there are examples that they cause cancer in humans. Epidemiologic
Evidence presently available on the association of exposure to fossil fuel combustion products and cancer in humans is not adequate as a basis for quantitative risk assessment of specific fossil fuel combustion products in the ambient air.

Experimental studies by use of bioassays on fossil fuel combustion products, in conjunction with relevant chemical and toxicological information, could provide a basis for risk assessment, but the presently available data are not yet adequate for a quantitative evaluation of human risk from specific products. Such bioassays include whole animal bioassays for tumor induction in mammalian species, bioassays for the neoplastic transformation of mammalian cells in culture, mutagenesis bioassays and tests for chromosomal changes, DNA repair, etc.

Data from the above listed sources are considered to be of value for estimating cancer risk to man, in the following order: epidemiologic studies > whole animal carcinogenesis assays > cell transformation and mutagenesis assays > chemical analyses alone. It should be pointed out that the most useful information derives from a combination of these data sources.

A useful basis for human cancer risk assessment from these products could be provided by adequate combinations of whole animal bioassays and/or by cell transformation and mutagenesis bioassays, not just by themselves but in adequate combination with animal bioassays and/or human epidemiological or laboratory studies.

A preliminary rough measure of carcinogenic potential can be suggested—in the absence of more adequate data—by a positive result of well designed single-animal carcinogenesis bioassays or by a positive result of combinations of in vitro tests, e.g., mutagenesis and/or cell transformation; such results, however, should be used with caution and in association with data on the chemical characterization of test materials.

Quantitative risk estimates using a linear non-threshold dose-response relationship to give a plausible upper-limit estimation may be useful in the choice of regulatory strategies for fossil fuel combustion products when derived from a sufficiently wide data base.

Recommendations

In vitro bioassays should be used for screening purposes, whenever appropriate and with due caution, to provide guidance on the comparative carcinogenicity of fossil combustion products, particularly where a series of engineering alternatives for the control of emissions are under consideration. In issues involving major long-range decisions it will be important to use the combined results of animal bioassays and short-term in vitro tests and whenever possible, epidemiological evidence.

For major decisions involving regulations, quantitative risk assessment should be considered for use, to the extent feasible, and with appropriate attention to its limitations.

In all circumstances, the inferences on human cancer risk from combustion products of fossil fuels as also from other environmental sources should be done by keeping in mind the environmental and biomedical relevance of the data.

Epidemiology*

The epidemiologic data relating air pollution to lung cancer are mostly derived from descriptive investigations carried out in the 1950's and 1960's, which have used vital statistics to contrast rates of lung cancer mortality in men and women, in urban vs. rural regions, and between migrants from different countries with different lung cancer mortality rates. In general, the random distribution of potential confounding factors has been assumed. However, cigarette smoking and occupational exposures are not randomly distributed, and these factors, which have far greater impact than air pollution on lung cancer rates, make interpretation of the available data uncertain.

In the absence of fully adequate epidemiological data on air pollution, complementary data sources have been used to estimate the potential cancer risks associated with exposure to combustion products. These include studies of cigarette smokers (19), coal gas workers (20,21), workers exposed to coke oven emissions (22,23), roofing tar emissions (24), and aluminum smelter emissions (25). In addition, combustion emissions and polluted ambient air contain potentially carcinogenic compounds that may cause cancer in other organs than the respiratory tract.

Although few studies have been directed specifically towards assessing carcinogenic effects of all automobile gasoline or diesel exhaust, two investigations have been cited in a number of recent reviews (3,26). The first of these studies was conducted in railroad workers exposed to diesel exhausts. This study suffers from the short period of time between the onset of exposure and the assessment of outcome. The second, conducted in London transport...
workers, suffers from not having smoking habits assessed and considering lung cancer only among workers who were in active service. Thus, conclusions about the study, although extensively reviewed, are uncertain.

As has been true for the last 25 years, specific statements at this time about the magnitude of the attributable risk of lung cancer from air pollution cannot be made with certainty. At a symposium at the Karolinska Institute in Stockholm in 1977, a conclusion was reached that “Combustion products of fossil fuels in ambient air, probably acting together with cigarette smoke, have been responsible for cases of lung cancer in large urban areas, the numbers produced being of the order of 5-10 cases per 100,000 males per year” (27).

Doll and Peto (15) prepared an extensive review of the causes of cancer in the United States in which they cited the Karolinska Institute Symposium finding, noting that “These crude estimates probably provide the best basis for the formation of (regulatory) policy.” They interpreted this rate as corresponding to approximately 10% of lung cancer in big cities or roughly 1-2% of all cancer for the country as a whole for age groups up to 65.

Efforts to make projections for the future have been based on attempts to identify indicators for past and current levels of pollution from fossil fuel combustion. It was generally agreed that improvement in ambient air quality has been associated with a reduction of health risks. However, it appears that neither BaP, which has declined by perhaps a factor of ten over the last few decades, nor total suspended particles, which have generally been reduced by a factor of no more than two, are adequate to be used alone as quantitative indicators for potential carcinogens in the ambient air. Thus, at this time there is no definitive way to estimate how changes in recent decades in ambient air contaminants have affected or will affect cancer rates.

For epidemiological studies to be useful in the future, they will have to be part of multidisciplinary collaborative studies. Subpopulations identified as potentially at risk will have to be studied intensively, both in terms of exposure and outcomes using state of the art industrial hygiene and analytical chemistry techniques for defining exposures. As part of the outcome assessment, detailed smoking histories must be obtained, including an establishment of an accurate lifetime history of smoking. Such studies will be extraordinarily difficult and expensive to carry out and, therefore, they must be carefully planned and financed with long-range commitments and might well be part of a larger effort directed towards more generalized goals. Nevertheless, such studies should have a high priority.

Conclusions

In spite of a great deal of uncertainty in the epidemiological evidence, the meeting reaffirmed the conclusion of the 1977 Stockholm symposium (27) that combustion products of fossil fuels in ambient air, particularly in connection with smoking, have been responsible for cases of lung cancer in humans, and found no reason to substantially change the numerical estimate.

At the present time there is no way to quantitate how changes in air pollution levels may have reduced mortality from lung cancer because there has been a lack of a completely reliable environmental indicator of air pollution carcinogenicity.

Recommendations

To be useful in the future, epidemiological studies should be a part of multidisciplinary collaborative efforts. It is imperative that particular attention is paid to a consistent estimation of exposure, using environmental and biological indicators. These studies will generally require long-term commitment of staff and material resources.

Chemistry*

Combustion processes in both mobile and stationary sources produce a large number of gaseous and particulate primary pollutants. Additionally, secondary pollutants are formed through complex atmospheric processes and thus add to the total air pollutant burden. The major gaseous pollutants CO, NO2 and SO2 are known to produce acute and chronic noncarcinogenic health effects. Concern for lung cancer has directed much attention to ambient levels of combustion generated particulate matter. By the late 1970s it was well established that such particles are in the respirable size range and their organic extracts contain chemically identified mutagens and carcinogens. There was growing concern that the identified chemical constituents did not account for all measured mutagenicity and tumorigenicity in ambient air samples.

During the past two decades there has been a substantial improvement in analytical chemistry techniques. These techniques have allowed detailed characterization studies of emissions from combustion sources in which literally hundreds of individ-

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ual compounds have been identified. Modern instrumentation has also allowed an identification of atmospheric transformation products and their environmental sinks. Moreover, the identified compounds can be quantitated at very low concentrations. The availability of short term bioassays, such as the bacterial mutagenesis assays, has led to bioassay directed fractionation and analysis. Such advances coupled with increased liaison between chemists and toxicologists have been used to identify biological active compounds and to determine the contribution of these compounds to the total mutagenicity of a fraction or extract (28).

Automotive Emissions

Combustion engines emit a variety of gaseous and particulate pollutants including many substances present in the fuel and their partial combustion products. Some of these compounds are mutagenic and/or carcinogenic.

Particles from combustion engines are usually less than 2 μm (29-31) in size. The diesel engine has been of special concern because its particulate emissions are an order of magnitude higher than those from a catalyst equipped gasoline engine (31).

Recent research efforts have particularly focused on diesel particles. These studies show that the extractability of standard compounds from diesel particles generally is satisfactory (80% or higher). Fractionation of these extracts using HPLC retains the mutagenic activity to better than 96% (28) and using compound class fractionation by liquid–liquid partitioning and silica gel chromatography resulted in 72–97% recovery of the mutagenic activity (32).

The extractable organics vary considerably, but are usually found to constitute 5–50% of weight of the submicron particles in diesel exhaust (33).

Until a few years ago it was generally believed that PAHs adsorbed on the particles were primarily responsible for the potential carcinogenic effects of car exhaust emissions. Bioassay directed fractionation and characterization studies have shown that this is not the case for diesel emissions. The most mutagenic components seem to be substituted PAH found in the moderately polar fraction of particle extracts (33-36). Such mutagenic PAH derivatives include compounds with hydroxy, ketone, quinone, carboxy aldehyde, acid anhydride, dihydroxy, and nitro substituents. Recently, attention has been focused on nitration of PAH since this may give rise to highly mutagenic compounds such as dinitropyrenes, which have been identified in car exhaust particles. Several independent investigators (33,37-40) have reported that nitrated PAHs (e.g., 1-nitropyrene) account for a significant portion of the direct acting mutagenicity of diesel particle extracts in Salmonella (TA 98). Another approach to identification of the mutagenic components of diesel particle organics has employed direct estimation of the mutagenic contribution from chemical analysis of the composition of the organics together with bioassay studies of identified compounds. Using this approach together with the human lymphoblast gene mutation assay has shown that for one diesel sample a substantial portion of the mutagenic activity could be accounted for by the presence of fluoranthene and 1-methylphenanthrene concentrations in the organics (41). These studies taken together suggest that both nitrated and methylated PAH make important contributions to the mutagenicity of diesel particle organics.

Gasoline engines produce much less nitrated PAHs than diesel engines (38,42), and the compounds responsible for the mutagenicity of gasoline engine emissions are for the most part unknown. Earlier studies of gasoline emissions coupled chemical characterization with rodent tumorigenesis studies (43). These studies show that the PAH may be the major class of chemicals contributing to rodent tumori-

| Table 1. Emissions from combustion engines.* |
|---------------------------------------------|
|                               | LPG | Gasoline, led. | Gasoline, catalyst | Gasohol* | Diesel | 95% MeOH |
|-----------------------------|-----|----------------|---------------------|-----------|--------|----------|
| Particulates, mg/km        | nd  | 50-100         | 5-10                | nd        | 750-1500| nd       |
| Benzene, mg/km             | < 1 | 50-150         | 1-15                | 50-150    | 10-20  | < 1      |
| Ethylene, mg/km            | 75-100 | 75-100         | 5-10                | 75-100    | 25-75  | 10-15    |
| Formaldehyde, mg/km        | 20-40 | 20-50          | 1-3                 | 30-60     | 10-15  | 100      |
| Benzo(a)pyrene, µg/km      | < 0.1 | 1-10           | 0.1-1               | 1-10      | 1-10   | < 0.1    |
| Methyl nitrite, µg/km      | 100-300 | 100-300       | 10-50               | 100-300   | 100-300| 5-6 x 10^2 |
| PAH, µg/km                 | 2.9 | 35-170         | 3                   | 35-170    | 500-1000| 2-9      |

*Data of Stenberg et al. (12) and Egebäck (49).

*Applies to either methanol (15%) or ethanol (23%) blended gasoline.

Sum of 15 PAHs (49).

Not determined.
genic activity although this class does not account for all the potential carcinogenicity of these emissions.

Particulate emissions collected from dilution tunnels on filter media appear to be representative of what is emitted to ambient air (44). However, most available data refer only to sampling conditions which reflected comparatively high dilution temperatures (= 40°C). There is a lack of information concerning the distribution of organics between gas phase and particles in the emission, when diluting at low temperatures < 0°C.

Combustion engines emit a large variety of gaseous compounds, including NO₂, CO, and low molecular weight organics. Table 1 illustrates some emission data for major organic emissions, including ethylene, formaldehyde, benzene, methyl nitrate and PAH. It is necessary to emphasize that these selected components may not be the most significant for anticipated adverse health effects on humans. The diesel engine clearly emits much larger quantities of particulate matter than a comparable gasoline engine. Total hydrocarbons (THC), particle mass (and the Ames activity) in reverts/km show relatively little variation with driving cycle (44, 45). Cold-temperature operation increases the organic emissions of gasoline-fueled passenger cars (46,47) but seems to have less influence on diesel organic emissions (48,49).

Coal-Fired Power Plant Emissions

Unlike motor exhausts, the primary particulate emissions from coal fired power plants under optimal conditions are generally derived from clay or siliceous minerals and contain relatively low concentrations of organic compounds. (For suboptimal combustion conditions, see below.) Further differences between motor exhaust and coal combustion emission relate to particle size distribution and physical properties. There are three distinct particle size modes for coal combustion aerosols (50). The supermicron mode (> 5 μm) generally reflects mineral agglomeration; the micromode (1-2 μm) is the result from the size distribution of the clay minerals associated with coal; and the submicron mode (< 0.5 μm) is the result of both gaseous cursting of aluminosilicate and homogeneous gas phase nucleation with subsequent agglomeration. The final size distribution of fly ash aerosols is strongly influenced by the particle abatement technology and combustion conditions. Because the efficiency of particulate capture is inversely proportionate to particle size, emissions from a modern coal-fired power plant are predominantly in the respirable mode (51).

All the generally accepted or strongly suspected human metal carcinogens (As, Ni, Cr, Cd, Be) are present in coal and are released to a certain degree with the flue gas (50,52,53). Most studies indicate that the most volatile elements (or their oxides), Cd, Zn, Se, As, Sb, W, Mo, Ga, Pb and V, display the greatest concentration dependence on particle size due to condensation on fly ash surfaces. The least volatile elements do not display a strong particle size dependence. It should be pointed out that some volatile elements such as Hg and Se may be emitted to the atmosphere in the vapor phase, without condensation on fly ash surfaces.

Coal combustion also releases 222Rn as a gas, and 235U, 238U and 232Th and their decay products in the particulate phase. 210Pb and 210Po are enriched in the smallest particles in the fly ash (50).

Despite the extensive literature on the elemental composition of coal fly ash (52), there exist few data on the valence state and chemical form of the elements. Hence, estimation of toxicity and bioavailability is difficult. One may expect, however, that the surface-associated elements will tend to be biologically available.

There is a dearth of information of the organic composition of coal fly ash. Generally the solvent-extractable organic composition of coal fly ash from modern pulverized coal plants is quite low. The total emissions of organic substances from coal fired boilers are dependent on the combustion efficiency. Large power plants fired with pulverized coal generally have efficient combustion and emit very small amounts of organic compounds whereas smaller boilers are often less efficient and produce greater concentrations or organic effluents (50). A variety of PAH has been identified in emissions from coal combustion, particularly under suboptimal combustion conditions. This includes benzo(a)pyrene, dibenz(a,h)anthracene, benzo(a)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benz(a)anthracene, chrysene, fluorene, fluoranthene, naphthalene, and anthracene (11). Similarly, gaseous emissions of organic compounds include ethylene, benzene, toluene, and a variety of alkanes (11). Laboratory studies have demonstrated organic chemical interactions with fly ash surfaces that can stabilize against or catalyze chemical decomposition (54). Little is known, however, about the environmental significance of the laboratory studies demonstrating organic chemical interactions with fly ash surfaces.

Ambient Air

Organic compounds present in the ambient air may be either anthropogenic or originating from natural sources. In urban air, the anthropogenic
sources will dominate. Present in the atmosphere are also reactive copollutants, like NO\textsubscript{x} and SO\textsubscript{x}, and other reactive compounds secondary formed in photochemical reactions. Adding to the complexity is that the emitted compounds will thus not only be differently distributed between gas phase and particulate matter compared to the conditions at the time of emission. Furthermore, due to the presence of reactive copollutants some of them may also be chemically transformed during transportation in the atmosphere.

Several metals known to be human or experimental carcinogens are present in ambient air particulate matter (52). Characterization of these elements is important since, for several metals, interactions with the effects of organic carcinogens or other metal carcinogens have been found (50,55,56).

A large number of volatile organic compounds, primary (P) as well as secondary (S) pollutants, has been identified in urban air (13,54,57-60). These compounds include classes such as alkanes (P), alkenes (P), alkynes (P), aromatic hydrocarbons (P), tetraalkyllead (P), phenols (P and S) and peroxyacetyl nitrates (S).

The photochemical conversion of gas phase materials in the atmosphere to form particles can account for a major portion of the particulate matter in certain urban atmospheres such as Los Angeles (13,61,62). Many of the compounds formed are nitrated hydrocarbons, aldehydes and peroxides for which little mutagenicity information is available.

Particle-associated organic compounds (54,63,64) have also been identified in ambient air, including compound classes such as aliphatic, aromatic and halogenated hydrocarbons, carboxylic acids and PAH. The mutagenic and carcinogenic effects of airborne particles have been demonstrated. Since these show a direct mutagenic activity, compounds other than conventional PAH must be present. So far heterocyclic aromatic compounds, quinones and mononitro-PAH have been identified in airborne particulate matter and several others like oxidized PAH (such as lactones) are probably present (13,54,65). However, only a minor part of the mutagenic components in samples of airborne particulate matter has been determined so far. Further efforts are needed to identify the unknown compounds. Particularly, the polar fraction of urban air particulate seems to contain biological active compounds (13,66) which are not identified.

A few decades ago urban atmospheric pollution often was associated with high concentrations of smoke and sulfur dioxide. While the air during the sixties and seventies has increased in transparency in winter time in e.g. cities in England, it has appeared to grow more turbid in the summer especially in rural regions (67,68). Today, photochemical air pollution is a common phenomenon, even in the Scandinavian countries (69). This increase in the occurrence of secondary pollutants is closely linked with changes in anthropogenic activities implying increased emission rates of nitrogen oxides. Nitrogen dioxide appears also to be a key component in transformation and formation reactions of genotoxic air pollutants in winter (54). Humans may, therefore, be exposed to different mixture of pollutants today than, e.g., in the fifties.

Benzo(a)pyrene (BaP) is one of the most frequently measured constituents of air pollution. Data from the National Air Surveillance Network in the U.S. suggest a considerable decline in the BaP concentration of urban atmospheres, during the period 1966 to 1975. The average BaP concentrations declined from an annual median value of 3.2 ng/m\textsuperscript{3} in 1966 to 2.1 ng/m\textsuperscript{3} in 1970 (70) and to 0.5 ng/m\textsuperscript{3} in 1975 (71). This decline is believed to be due primarily to the decrease in coal consumption for house heating, as well as improved disposal of solid waste and restrictions on open burning (70,71). The decline in BaP concentrations may also have resulted from reduced emissions from industrial sources.

A decline in the BaP concentration has also been observed in other countries. The annual average BaP concentration in London decreased from 3.9 ng/m\textsuperscript{3} in 1962-1963, to 1.0 ng/m\textsuperscript{3} in 1972-1973. The data show a 90% reduction over the past 25 years (72).

It should, however, be stressed that a reduction in the BaP level does not necessarily mean a reduction in the potential health hazard, since the spectrum of pollutants has also changed with time.

### Chemical Transformations

Model experiments in simulated atmospheres suggest that PAH and other aromatic compounds are transformed in the atmosphere (13,54). The dominant pathways will depend upon seasonal and meteorological conditions, e.g., light intensity, humidity, temperature as well as the presence of reactive primary and secondary copollutants (13,54). For example, in winter in the Scandinavian countries the major recognized reactions of PAH appear to be those with nitrogen dioxide and nitric acid (54). Reactions with nitrous acid and sulfur oxides may also occur. At higher solar intensities, photo-oxidations as well as reaction with photochemically generated pollutants such as ozone and peroxyacetyl nitrate may also be important (54).

Reactions with free radicals known to be present in the atmosphere, especially the hydroxyl radical, must also be considered although only few data are available regarding such reactions (54).

PAH deposited on a glass fiber filter or on the
surfaces of particulate matter react with NO$_2$. The reactions are catalyzed by nitric acid and vary with exposure to actinic light. While the reaction mechanisms are not known, the reaction rates of different PAH appear to follow at least roughly theoretical expectations (13,54).

Model experiments have demonstrated that BaP is reactive towards ozone at levels observed in ambient air yielding quinones, dialdehydes, dicarboxylic acids, ketocarboxylic acids and dihydroxy derivatives as well as other compounds.

Photooxidation reactions transform at least some PAH in model experiments to direct mutagens. For example, pyrene forms a lactone upon photoexcitation (13). However, detecting and identifying labile species such as lactones and endoperoxides etc. is difficult because they undergo reactions easily under typical conditions used in workup of the sample.

Virtually nothing is known about either the rates of mechanisms or reactions of particle-associated PAH with radicals. The hydroxyl radical is present in both “clean” air and photochemically polluted air (54,73). It is very reactive, transforming monoaromatics in gas phase reactions. Reactions of gas phase and particle-associated PAH with radicals is clearly a field which requires attention in the near future. Reactions with nitrogen trioxide may be important sink processes for certain derivates of PAH (e.g., phenolic PAH).

Extrapolation of data from simulated atmospheres is a difficult operation, since our understanding of the fundamental processes occurring in most cases is rather poor. At least three types of reactions must be considered for particle-associated components: (1) reactions between reactive gaseous components and adsorbed components, (2) reactions between reactive species dissolved in the liquid film on the particles and the adsorbed components, and (3) reactions between adsorbed species.

Recent data (11,13,54) on the presence of genotoxic components in samples from stack and exhaust gases and in samples of particulate matter in ambient air indicate that chemical processes occurring during transport in the atmosphere and/or during sample collection may cause changes in the composition.

Model experiments suggest that these reactions may transform noncarcinogenic/nonmutagenic PAH to biologically active components or the reverse; hence, the genotoxicity may be altered.

**Sampling and Artifact Formation**

As motor vehicle and power plant exhaust is discharged into the atmosphere, a series of chemical and physical transformations occur during air dilution. This is particularly well studied for PAH (74). The goal of sampling system and strategy design is to preserve the chemical composition, phase distribution and particle size of emission in the ambient air. Sampling for biological testing involves special problems since large sample quantities are needed and the important carcinogenic and mutagenic compounds often are present in minute concentrations. Therefore, special attention has been paid to two features of sampling design: facility of large volume sampling and avoidance of artifactual synthesis during sampling.

Diesel particle sampling has been comparatively well studied. Dilution tube techniques appear to reflect well the physical and chemical character of particulates emitted to the atmosphere. Nitration artifacts are known but can be minimized by low sampling temperature, limiting sampling time to 30-40 min and limiting NO$_2$ to less than 3–5 ppm (28).

Gasoline particle sampling is not as well studied, but it is expected that the distribution between the gas and particle phase is not similar to what is found for diesel emissions. For catalyst vehicles the phase distribution of organic substances is unclear. Nitration artifacts are not expected with gasoline engines because the exhaust is low in NO$_2$.

Gas phase collection devices have been designed and used to roughly estimate the contribution of gas phase emissions to Ames mutagenicity. Devices used so far include raw exhaust condensers (75), dilute exhaust cryocondensers (76) and porous polymer traps (28,77). Limited evidence suggests that these devices might give comparable results with diesel exhaust, but with leaded gasoline and catalyst vehicles there are discrepancies among sampling systems which need further study for resolution.

With regard to both vehicles and utility boiler emissions, there are serious concerns about transfer of hot exhaust to collection devices, since recovery studies have shown low efficiency for certain species.

Sampling of flue gases from coal- and oil-fired utility boiler exhausts for mutagenic substances should involve the concerns expressed for car exhaust. The performance of the systems currently in use (with respect to phase distribution and mutagenic artifacts especially) is not well known. Hot gas filtering with porous polymer trapping probably does not simulate plume conditions and special techniques for particle separation may be better.

Ambient air collection techniques are even less well understood than source sampling. Filtration can involve organic artifacts from reaction of co-pollutants such as O$_3$ and NO$_2$ with PAH and there is also evidence for volatilization of organics in long-term sampling (13,74).
Conclusions

Direct mutagens, as well as promutagens, are found in respirable particles collected from ambient air and exhaust emissions from diesel and spark engines, as well as coal-fired combustion sources. Some exhaust gases are known mutagens and/or carcinogens or precursors. A number of other unknown chemical species directly mutagenic in the Ames' assay are present in extracts of ambient particulate matter and in exhaust particulates from diesel cars.

Mutagenic and chemical data on some sources of combustion generated emissions are not available at present. Diesel engines produce up to 100 times more respirable particles per km than a well-functioning gasoline engine with catalytic control. With respect to gaseous emissions, diesel engines emit relatively more of the total NOx as NO2 contributing to the formation of nitroarenes.

Diesel vehicles emit about six times as much organic, solvent-extractable particle mass as leaded gasoline vehicles and 20 times more than do in-use catalyst cars. Diesel organics are substantially higher in nitroarenes than gasoline organics, which again are higher in PAH. Vapor phase (> C7) organic mutagens are somewhat controversial. However, using the maximum estimate available, the vapor phase from diesel cans can add only about 20% to the mutagen yields from the particulate phase. In leaded gasoline cars, the vapor and particulate phase mutagen yield may be about equal. The composition of C2–C7 gas phase emissions is well defined. However, the mutagenicity of the C2–C7 species is not well known.

Catalyst technology offers about a factor of four advantage over leaded gasoline in PAH emissions for in-use cars. Coal-fired particulate emissions have been less well studied, but are often known to contain mutagenic compounds. Organic content on fly ash is frequently as low as 0.01%. Organic compounds identified include PAH. Vapor-phase organics constitute the major amount of the organic emissions. Inorganic substances of particular importance include heavy metals and radioactive elements. Furthermore, the inorganic substrate differs significantly in terms of physical and chemical properties from the carbon base characteristic of diesel particles.

Suboptimal operating condition for coal and wood combustions may result in significantly increased emission. Automotive emissions do also vary, in some cases up to a factor of 10.

Studies using simulated atmospheres have shown that some three- to five-ring PAHs react readily with gaseous copollutant such as NO2 and O3. These products have been identified in combustion generated particles. Additional reactions with a variety of other co-pollutants seem like.

Combined chemical-microbiological investigations have proved to be a powerful tool for chemical analysis. Using this approach it has been shown that up to 40% of the total direct mutagenic activity of certain diesel particulate emissions are mono- and dinitroarenes. While the concentrations of such nitroarenes are relatively small, their contributions to the total mutagenic activity of the particular samples are large.

Major contradictions appear in the literature describing the mutagenicity and carcinogenicity of purported “pure” compounds. However, subsequent analyses demonstrated the presence of small amounts of highly mutagenic trace impurities.

Chemical transformation reactions depending on ambient conditions may increase or decrease the mutagenic activity or change the mutagenic characteristics.

BaP has historically been used as an indicator of general air pollution. BaP may be subject to atmospheric transformations which severely limit the utility of BaP data in risk assessment.

Chemically and biologically significant differences exist between the composition of gaseous and particulate samples collected over short-term (e.g., 1 hr) vs. long-term (e.g., 1 day, 1 week) periods.

A variety of methods have been employed for sampling direct emissions from both stationary and mobile sources, as well as ambient air. While they yield results which are generally in qualitative agreement, serious quantitative differences can exist for both gases and particulate emissions. Automotive emissions collected from dilution tunnels generally appear to be reasonably representative of emissions into the environment.

Chemically and/or microbiologically significant artifact formation can take place in the sampling, extraction, separation and analysis of both primary and secondary particles.

Only a few reports on improved sampling techniques for coal-fired boilers and power plant emissions have emerged recently. Systems taking into account similar devices to those for motor vehicles exhaust should be further developed and evaluated.

Recommendations

Elucidation of the chemical nature and concentrations of presently unidentified mutagens and carcinogens in combustion generated particulate emissions as well as in ambient aerosols should have a high research priority. In these studies great care should be taken to assure the precision and accuracy of sampling and analytical techniques, as well as the subsequent bioassays.
The products, rates and mechanism of the reactions of PAH and other combustion generated organics with a variety of copollutants (e.g., free radicals and singlet oxygen) should be determined under a variety of conditions of temperature, light intensities, surface substrates, etc.

Fundamental and applied studies relating to the extractability of mutagenic/carcinogenic substances from particulate mixtures are needed.

Quality control and quality assurance programs for both biological and chemical evaluation of potential mutagenic and carcinogenic substances identified from combustion-generated particulate emissions should be developed on both national and international levels. This should include the establishment of a cooperative international group dedicated to supplying such pure compounds as suggested.

The mutagenic and/or carcinogenic impact of gaseous species emitted in combustion processes, or subsequently formed in the atmosphere, such as formaldehyde and nitrous acid, should be explored. Concurrent with these studies, measurements of gaseous levels in ambient air should be carried out to estimate population exposure.

Sampling techniques for both exhaust emissions and ambient air should be further refined with special reference to minimize artifacts as well as to expand the usefulness of these techniques for sampling over a wide range of environmental conditions (e.g., temperature, altitude, relative humidity, presence of copollutants).

A more comprehensive and reliable emission inventory of mutagenic combustion-generated emissions, both gaseous and particulate, should be performed. Target areas include leaded gasoline-fueled vehicles and residential combustion of wood and coal.

The effects of degradation with time and use of engine systems, boilers, etc. on emissions of mutagenic and carcinogenic materials should be explored in more detail. The efficiency of catalytic and other control devices for gasoline fueled vehicles should be further investigated under "real-world" operating conditions.

Research of respirable ambient particles should be performed with special attention directed to short-term collection (approximately 1-3 hr) to provide an indication of peak mutagen doses. Additionally, there is a need for more sampling of gaseous and particulate mutagens at their point of release into the atmosphere.

Identification of chemical indicators of carcinogenic compounds for the general air pollution level should be strongly encouraged. However, BaP monitoring should also be continued in the event that a technique for correlating the historical BaP data base with newly developed tracer is found.

Transformation of gas-phase two- to four-ring PAH in the atmosphere may lead to particle-associated genotoxic components. Studies should be carried out to evaluate the life-time of gas phase PAH in ambient air and to identify the transformation products.

Metallic compounds constitute major and minor components of particulate emissions from coal and oil combustion, respectively. Because the biological activity of metals is determined by their chemical form, further studies of chemical speciation are required.

**Short-Term Bioassays**

The fundamental similarity between the genetic material in all cells is the scientific basis for using short-term carcinogenesis and mutagenesis bioassays. Short-term bioassays are conducted with microorganisms such as bacteria and yeast, plants, insects, isolated mammalian cells, and in some cases whole animals to detect a substances genotoxicity. Different biological endpoints related to genotoxicity are detected by short-term bioassays including: gene mutation, DNA damage and repair, chromosomal effects, and morphological cell transformations.

Short-term bioassays have been useful in the screening and evaluation of complex mixtures, particularly combustion emissions in order to indicate emissions that are potentially mutagenic or carcinogenic and that should be evaluated in confirmatory and possibly long-term whole animal assays; direct the fractionation and identification of hazardous components and specific chemicals in complex mixtures; and compare the relative biological activity of similar emissions from alternative sources, fuels, control technologies or operating conditions.

**Motor Vehicle Emissions**

Short-term bioassays have been recently used to evaluate the mutagenicity and potential carcinogenicity of the extractable organics from motor vehicle particle emissions. The Ames *Salmonella typhimurium* plate incorporation assay demonstrated that both diesel and gasoline particle organics are positive in strains TA 1537, 1538, 98, and 100, but negative in TA 1535 (34). This strain specificity indicates that the mutagenic components in motor vehicle particulate emissions cause frame shift, but

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*This section was prepared by a Workgroup chaired by J. Lewtas (Health Effects Research Laboratory, EPA, Research Triangle Park, NC, USA). Other members of the Workgroup were: U. Ahlborg, I. Alfheim, O. Andersen, B. Andon, J.-Å. Gustafsson, G. Löfroth, C. Ramel, U. Rannug, T. Sugimura, R. Toftgård and K. Victorin.*
not base substitution. Diesel particle extracts usually have shown a higher mutagenicity in the absence of an exogenous metabolic activation system (S9) while gasoline particle samples have the opposite tendency, indicating a somewhat different spectrum of mutagenic compounds (78). Bioassay-directed fractionation and characterization studies (discussed in the chemistry section) have shown that a significant portion of the mutagenicity of the diesel samples is due to nitro-PAH while the mutagenicity of gasoline samples appears to be due to the PAH content to a greater extent. Although the gasoline (both catalyst and noncatalyst) particle extracts are more mutagenic per microgram of organics than the diesel extracts, the diesel engine clearly emits much greater total quantities of particulate and associated organic matter than a comparable gasoline engine. The mutagenic activity of diesel vehicles is therefore much greater than gasoline vehicles when compared on the basis of distance driven (79). Studies have been conducted to evaluate the effect of various parameters (fuels, engines, driving cycle, temperature, catalysts, etc.) on the mutagenicity of the emissions by using the Ames S. typhimurium assay (80).

The extractable organics from both diesel and gasoline particle emissions have been shown to be mutagenic in several forward mutation assays in mammalian cells (81-83). The most extensive mammalian cell mutation data base on different diesel, gasoline, and comparative samples is in the L5178Y mouse lymphoma cell assay at the thymidine kinase (TK) locus (81). In this assay most of the diesel samples were mutagenic in the absence of S9, whereas the gasoline samples exhibited increased activity in the presence of S9. The extractable organics from diesel emissions are also mutagenic in Chinese hamster ovary (CHO) cells at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus (82), in BALB/c 3T3 cells at the ouabain locus (81), and in human lymphoblasts at the thymidine kinase (TK) locus (16). The diesel particles themselves were found to be both phagocytized and mutagenic in CHO cells (84). It is possible that NOx-PAHs may contribute to the mammalian cell mutagenicity of diesel particles. Di- and trinitropyrenes were mutagenic in Chinese hamster lung cells using diphtheria toxin resistance as marker without S9. However, 1-nitropyrene under the same conditions did not demonstrate any mutagenic activity (10).

Several DNA damage assays in both yeast and mammalian cells have shown activity with both diesel and gasoline emissions (81). These assays include detection of sister chromatid exchanges (SCE) in CHO cells and mitotic recombination in Saccharomyces cerevisiae. Chromosome aberrations were observed in CHO cells and human lymphocytes treated in vitro with diesel organics (81). No studies have been reported on the effect of automobile emissions on the spindle fiber mechanism and disjunction of chromosomes. However, alkyllead compounds have been shown to be very potent spindle inhibitors giving rise to nondisjunction of chromosomes (85).

Extracts of motor vehicle particulate emissions have been successfully assessed in two cell transformation assays; the BALB/c 3T3 mouse embryo cell assay (a direct measure of cell transformation) and Syrian hamster embryo SA-7 viral transformation system (an indirect measure of cell transformation) (81). In both cell types there is significant evidence for increased oncogenic transformation with the Syrian hamster embryo system giving good dose-response relationships. The results in these systems which measure an effect closer to cancer in vivo than mutation assays provide strong evidence for the carcinogenic potential of the extracts. However, the assays do not provide sufficient reliable quantitative prediction of the carcinogenic potential of the extracts, or particles, in vivo.

During in vivo exposure, diesel exhaust did not have any effect on micronuclei or SCE in bone marrow or on point mutations in specific locus, dominant lethal and heritable translocation tests in the mouse (81,86). Likewise, micronucleus assay in the Chinese hamster and the fetal liver SCE assay in the Syrian hamster were negative. One positive effect was, however, reported for Syrian hamsters showing an increased incidence of lung cell SCE. With collected particles, the SCE assays were positive both in bone marrow cells of mice and in lung cells of Syrian hamster after in vivo exposure. The extracted organics from diesel particles were positive in the micronucleus assay in mice and hamsters and SCE's in hamsters in lung cells and fetal liver (86).

Measurements have been made to determine the distribution of Ames mutagenicity between the gas and condensed phases. Two methods, cryogenic condensation (76) and absorption on XAD-2 polymers (33,77), have been used. During preparation of the samples for short-term bioassay some of the gas phase compounds may be lost by evaporation. For gasoline exhaust the vapor phase contribution to the total mutagenicity on TA 98 has been reported to be less than 10% (33) or not exceeding "background levels" (77) when using XAD-2 traps. Results obtained (78) by using cryogenic traps, however, showed a vapor phase contribution of 10-20% in the presence of S9 and a contribution of up to 70% in the absence of S9 on the same tester strain (TA 98). Data on diesel exhaust show that between 10 and 20% of the observed mutagenicity of TA 98 can be
found in the vapor phase regardless of trapping technique (77,78). Catalyst-equipped vehicles showed no significant mutagenicity in the vapor phase (77,78). The data also suggest that the distribution between the mutagenicity of particles and vapor phase can vary considerably depending on engine and fuel.

Short-term in vivo bioassays with nonmammalian systems have been reported for both the total gaseous and particle emissions and filtered gaseous emissions from diesel and gasoline vehicles. The stamen hair mutation assays in Tradescantia plants have been reported as positive but weak in comparison to some urban atmospheres (81). Negative results for sex linked recessive lethals in Drosophila have also been reported for whole and filtered motor vehicle emissions (81) but, considering the possible role of PAH and PAH derivatives, it should be pointed out that these compounds are not efficiently activated by the mixed function oxygenase in this organism. BaP is thus negative in ordinary Drosophila sex-linked recessive lethal tests.

Coal-Fired Power Plant Emissions

Mutagenic components of fly ash appear to be both organic and inorganic compounds. The mutagens detected by the Ames' Salmonella assay are predominantly direct-acting frameshift organic compounds, resistant to photodecomposition, but which decompose with heating to 350°C (88). These mutagens appear to be concentrated in the finest, most respirable fly ash fractions. Inorganic acid-soluble mutagens have also been detected using mutagenicity test with the protozoan Paramecium tetraurelia, that engulf the particles (89). Some of the mutagenic activity detected with the Paramecium test could be extracted with HCl but this extract was not active in the Salmonella assay (89). It has been shown that the surface of the ash particles contains water soluble compounds that cause DNA damage in bacteria (90). The fly ash may thus contain inorganic mutagens that do not show up in the Ames' Salmonella mutagenesis assay. Neither class of mutagens has been chemically identified, although nitroaromatic hydrocarbons have been implicated as major contributors to the bacterial mutagenicity.

Comparison of the mutagenicity of particle emissions from coal-fired power plants to other combustion sources on a fuel consumption basis (e.g., revertants/g fuel) shows that large (25–600 MW) coal-fired power plants, when operating efficiently, have an extremely low mutagenic emission rate (66) based on the particle emissions. Technical difficulties have prevented measurement of the mutagenicity of the gas phase of coal-fired power plant emissions. Collection of large quantities of particle emissions low organic content of such samples. Mutagenicity not detected with the Ames Salmonella assay with several samples has however been reported as positive in the mammalian V79 hamster cells assay (11,53). The different response observed for bacterial and mammalian cell assays may be due to the more toxic inorganic components of the fly ash. In many of these investigations the samples have been taken under optimal conditions of operation. Under on- and off-conditions as well as under operating disturbances that give rise to an incomplete combustion, the mutagenicity has been shown to be much higher, particularly for fluidized bed coal combustors (11,91).

When sufficient quantities of fly ash have been collected at temperatures below 350°C such that the extractable organics can be bioassayed directly in the Ames Salmonella assay, the mutagenic activity of these organics is in the range observed for other combustion emissions. Comparison of the mutagenicity of large coal- or oil-fired power plants with smaller residential wood or oil combustion sources shows that the mutagenicity of the smaller residential units is generally many orders of magnitude greater based on mutagenicity/mass of fuel consumed (66,92).

Ambient Air

The mutagenic activity of organic extracts of ambient particulate matter in Salmonella typhimurium has been reported from several parts of the world. Industrialized and urbanized areas generally contain more mutagenic substances than rural areas although rural areas may be influenced by local transport of mutagenic airborne particles (65).

Only a minor part of the total mutagenic activity, as detected by the Salmonella assay, can be accounted for by PAH compounds requiring mammalian activation. Compounds not requiring this activation are, with few exceptions, chemically unidentified. The mutagenic activity in nitroreductase-deficient strains of Salmonella indicates that some of this activity is caused by nitro-substituted polycyclic compounds (66). Chemical fractionation of organic extracts of particles from ambient air has shown that the directly acting mutagens will be found predominantly in the fractions containing the most polar compounds and/or organic acids (66,93). Atmospheric reactions may increase or decrease the mutagenic potential of emitted compounds and cause a shift of the distribution of the mutagenic response in fractions of different polarity. Very few data are available on the mutagenic activity of gaseous organics collected from ambient air in microbial and mammalian cell tests. The short-term plant bioas-
say in Tradescantia, however, has been utilized to detect mutagenic gaseous compounds in ambient urban air (94).

The contribution from various sources to the mutagenicity of ambient air has been estimated by several investigators (37,66,95). All of these studies suggest that combustion sources in general are important. Residential heating, for example, may account for much of the increased mutagenicity observed in winter compared to summer. Motor vehicles are also a major source of mutagenic compounds in urban air (68) as evidenced by the differences observed between day and night and at low temperatures has been difficult due to the condensation of sulfuric acid under such conditions. No mutagenicity studies to date have been reported on ambient temperature collection of particles downwind of coal-fired power plants. A number of reported studies on the lack of mutagenicity of fly ash is due to the high collection temperature and extremely between street level and roof top level values measured for the mutagenicity of urban air.

Conclusions

The extractable organics associated with combustion particle emissions from motor vehicles, coal, oil and wood combustion are mutagenic in short-term bioassays. For motor vehicle emissions, the organics associated with particle emissions are genotoxic based on a variety of in vivo and in vitro short-term tests. These extractable organics from coal combustion and other stationary source combustion particle emissions (e.g., wood, oil, refuse, etc.) as well as ambient air have been found to be mutagenic in microbial tests but very little data in other bioassays is available.

Mutagenic compounds in combustion particle emissions and polluted ambient air includes PAH as well as oxygenated and nitrated PAH and unknown chemical species. There are differences with respect to the response in various strains and the S9 requirement for different sources. These differences can be utilized for characterization. Ambient airborne particulate matter has more mutagenic activity associated with the more polar compounds than most emission sources.

Some compounds in the gas phase, i.e., compounds noncondensable at ambient temperatures, either at the emission source or in the ambient air, are mutagenic. Further development of methodologic procedures will be required to determine the relative contribution of the mutagenicity of this gas phase to the total mutagenicity. At present the evaluation of the gas phase must be based on mutagenicity bioassay of pure gases identified by chemical analysis.

Comparison of relative particle associated mutagenic emission rates for mobile and stationary sources show some significant differences as follows. Diesel vehicles have higher mutagenic emission rates per kilometer than gasoline noncatalyst vehicles, which in turn have higher rates than gasoline catalyst vehicles. Residential wood stoves have higher mutagenic emission rates per joule than residential oil heaters. No significant difference in mutagenic emission rates per joule has been reported between coal and oil in similar utility boilers. The mutagenic emissions are dependent on the mode and efficiency of the combustion, and are generally low in well-operated units.

The high qualitative correlation between bacterial mutagenicity and animal carcinogenicity justifies the use of these short-term tests for the identification of potentially carcinogenic organic chemicals. Although reasonably good correlation between mutagenic and carcinogenic potency is found for certain classes of chemicals a general correlation cannot be expected for all chemicals (96,97). Within a series of extractable organics from motor vehicle particle emissions a good correlation was observed between bacterial mutagenic activity, mammalian cell mutagenic and transforming activity, and skin tumor initiating activity.

Recommendations

Mutagenic activity, as detected by short-term bioassays, is a useful environmental monitoring tool, especially for complex mixtures for which all of the hazardous components have not been identified. Investigation should be performed with the Ames Salmonella/microsome assay using several strains to evaluate the mutagenic level and in a gross manner the presence of certain classes of compounds present in various combustion emissions and ambient air. Selected samples should be evaluated in mammalian and other short-term bioassays in order to assess the activity in a variety of biological systems.

Some organic compounds in the vapor phase (C2-C7), i.e., compounds generally not collected by condensation or adsorbents at ambient temperatures (less than C7), either at the emission source or in the ambient air are mutagenic. Further development of methodologic procedures will be required to determine the relative contribution of the mutagenicity of this gas phase to the total mutagenicity. At present evaluation of the gas phase must be based on mutagenicity bioassay of pure gases identified by chemical analysis.

More research is needed on the mutagenicity of gaseous components of emission sources and ambient air. Research should include development of
bioassays for gas phase compounds and mixtures. Detection of tumor promoters in combustion emissions and ambient air may also be important in relation to human carcinogenesis. For this purpose, inexpensive and reproducible methods to detect tumor promoters are needed and should be developed.

The chemical nature of mutagens in ambient air should be studied further. Further analysis of the chemical components of ambient air may provide important information as to their generation through atmospheric conversion of combustion effluents or from unidentified sources of emissions. Studies on ambient air should, in addition to outdoor air, include indoor air inside residential homes and work places, since cigarette smoking and cooking for example have been shown to contribute to the organics in air to which humans are exposed.

The relevance of background mutagenicity data with air pollution samples in relation to human cancer risk, must rely on the correlation between the mutagenicity and carcinogenicity of the samples tested. Studies of the relation between mutagenicity in bacterial and other short-term systems on the one hand and animal carcinogenicity on the other with selected air pollution samples and pure carcinogens of relevance in this connection, should be given high priority in future research.

Efforts should be made to attempt to determine the relative importance of the mutagenicity of ambient air in relation to the total "mutagenic exposure" impinging upon the human (also mutagens in food, drinking water, etc.).

Further studies of the mutagenic and carcinogenic potential of metals in combustion products are required to better assess human health hazard of such products.

**Whole Animal Bioassays**

A triad of types of studies in (a) subcellular, cellular and tissue systems, (b) whole animals and (c) man is used to obtain an adequate assessment of the potential health effects of air pollutants on man. Since our ultimate interest is man, it is obvious that human data, when available, are most useful to assess human health risks. Unfortunately, emissions from motor vehicles or coal combustion are similar in many ways to pollutants released from other sources. Likewise, the diseases they are likely to produce are not unique to air pollutants. Thus, it has been difficult to obtain epidemiological data on the health effects of these particular emissions. The problem is especially confounded by the predominant role of cigarette smoking in producing pulmonary disease, including cancer.

In the absence of adequate human data, it is necessary to turn to test systems like short-term bioassays using subcellular elements, cells or tissues from laboratory animals or man, as described in the preceding report, or whole animal studies as will be discussed here. These studies can be conducted under rigorously controlled experimental conditions in contrast to the epidemiological studies. As a result, it is possible to assess the role of variables that are difficult or impossible to assess directly in man, thereby gaining added insight into the mechanisms underlying the diseases caused by air pollutants.

Whole animal studies have both advantages and limitations for studying the health effects of air pollutants. The advantages may be summarized as follows. In the whole animal, as in man, the multiple processes such as cell injury, repair, transformation and promotion under the influence of many host factors interact to yield observable disease such as lung cancer that may not be directly observed in isolated cells. By using serial observations it is possible to follow the pathogenesis of the diseases of interest. The method of administration of the emission or emission components can be chosen to come close (i.e., intratracheal instillation) or simulate (inhalation) the actual human exposure situation. The fate of inhaled materials, i.e., the deposition, retention in the respiratory tract and clearance from the respiratory tract to other organs or from the body, can only be evaluated in the intact animal.

The limitations may be summarized as follows. Whole animal studies are more expensive and take longer to complete than studies using simpler systems. Those factors, in turn, limit the number of pollutants or pollutant constituents that can be studied in whole animal assays. Because of limitations in the number of animals that can be studied and the life span of the species, it is usually necessary to utilize exposure levels many times higher than those typically encountered by man. (However, it should be noted that on a relative basis the exposure levels are at least as relevant as those used in most in vitro assays.) The induced diseases observed in laboratory animals may not always have direct counterparts in man, and vice versa. This is illustrated by our inability to yet identify a fully satisfactory animal model for induction of lung cancer by cigarette smoke despite its demonstrated carcinogenic effect in people.

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*This section was prepared by a Workgroup chaired by R. O. McClellan (Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM, USA). Other members of the Workgroup were: B. Holmberg, S. Nesnow, G. Nordberg, U. Saffiotti, W. Stöber, S. Takayama and J. Vostal.*
Within this report there are discussions of the utility of various whole animal assays for carcinogenicity and of whole animal studies for evaluating the fate and local effects in the respiratory tract of inhaled materials. These assays include the use of subcutaneous injection, skin application, intratracheal instillation and inhalation exposure techniques. In each area, the current state of our knowledge relative to emissions of automotive vehicles and coal burning power plants is summarized.

**Fate of Inhaled Material**

The predominant route of exposure to the emissions from motor vehicles and coal combustion is by inhalation. Therefore, an assessment of the fate of inhaled materials is essential. The deposition and interaction of gaseous components of emissions such as SO$_2$, NO$_x$, CO, and aldehydes are governed by the chemical characteristics of the gas and particularly its reaction with biological materials.

The particles emitted in the exhaust of motor vehicles are all of a respirable size ($29-31$). They have a high potential for deposition in the pulmonary region of the respiratory tract with lesser quantities deposited in the nasopharyngeal and tracheobronchial compartments. The deposition of radiolabelled ultrafine particles similar in size and shape to diesel exhaust soot has been studied in Beagle dogs. Approximately 25% of the particles were deposited in the pulmonary compartment and less than 10% in the nasopharyngeal and tracheobronchial compartments (98). These are considered to be good estimates for man until such time as similar data can be obtained in people.

Power plant emissions vary in particle size distribution and composition upon the fuel (for example, coal versus oil) and especially the emission control technology being used (51). Modern emission controls markedly reduce the particulate emissions, primarily by removing the largest particles. Most of the particles released to the atmosphere are of a respirable size. Their deposition can be predicted from knowledge of the size distribution of the particles and available data on particle deposition in man (99). Particles resulting from combustion are not considered to have properties uniquely different from those from other sources that will influence their deposition in the respiratory tract.

Particles that deposit in the nasopharyngeal and tracheobronchial compartments, which are mostly lined with mucus-overlying, ciliated epithelium, are rapidly cleared to the oropharynx and ingested. Due to the effective nature of the mucociliary clearance mechanisms, epithelial cells lining the conducting airways receive only a transient direct exposure to inhaled materials. The amount of cleared material of low solubility that is ingested may be substantial and the effects in the gastrointestinal tract must be considered. The extent to which the constituents of these ingested particles may be metabolically altered in the gastrointestinal tract is not known.

On the other hand, particles or absorbed compounds that are sufficiently soluble in body fluids may be absorbed in the nasopharyngeal and tracheobronchial compartments. Similarly, particles with these characteristics deposited in the pulmonary region may be rapidly absorbed, if sufficiently soluble in body fluids. Less soluble particles, however, will be engulfed by macrophages.

In general, the clearance of particles deposited in the respiratory tract is proportional to the total quantity deposited. However, with deposition of large quantities, the clearance rate from the pulmonary compartment is considerably reduced. Although deposited particles are rapidly phagocytized, the rate at which they reach the ciliated surfaces is relatively low so that they are cleared rather slowly via mucociliary action. Other macrophages containing particles as well as some free particles that have not been phagocytized may enter the interstitium. Some particles may reside in the interstitium for extended periods of time, while other particles may return to the alveolar space. This is a dynamic situation such that particles in macrophages may be recovered from the alveolar spaces by lavage even when several hundred days have elapsed between inhalation of the particles and lavage or washing of the lung (100). Particles may also enter lymphatic channels and be cleared to regional lymph nodes.

The retention half time of diesel particles in the lungs of rats exposed at low concentrations is on the order of a hundred days ($6,101,102$). Rodents generally clear other types of particles from the lungs more rapidly than is observed in the dog or man ($103,104$). Thus, it is possible that diesel soot particles will be retained longer in the lungs of man than of the rat. When larger quantities of diesel exhaust particles are deposited in the lungs of rats, either by exposure to high concentrations of exhaust or by prolonged exposure to more moderate concentrations, the clearance of the particles from the alveolar region of the lungs may be impaired. As a result, greater quantities of particles are retained in the lung for longer periods of time (hundreds of days) than would be predicted from studies of lower exposure concentrations ($6,101,102$).

The fate of organic compounds and individual elements, i.e., metals, associated with inhaled and deposited particles is not as well understood as the fate of the carrier matrix of the particles. The significant matrix differences among particles (ranging from fly ash that is primarily aluminosilicate to
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Two days, no mutagenicity was detected. It is noteworthy that nonspecific fluorescence in the extract was also lost. In vitro incubation of alveolar macrophages with diesel exhaust particles also resulted in loss of mutagenic activity and of 1-nitropyrene (111). The mechanism for loss of the mutagenic activity is unknown. The compounds responsible for the mutagenicity may have left the particles and been translocated to other tissues; they may have been metabolically transformed and still be present in a deactivated form, or they may have been metabolically destroyed.

In vitro studies have demonstrated a slower rate of release of organic compounds from diesel exhaust particles treated with saline, lung lavage fluids, or serum than with organic solvents such as dichloromethane (6). There is also an associated difference in the released mutagenic activity assayed in bacteria. Treatment of diesel exhaust particle extracts with serum or lung or liver cytosol decreases the detectable mutagenic activity (112). Subsequent treatment with protease partially restores the mutagenic activity, suggesting that binding of the mutagenic compounds to protein may be a factor in reducing the mutagenicity. The cytotoxic effects of diesel exhaust particle extracts on Chinese hamster ovary cells was also shown to be reduced by mixing the extracts with animal sera, lung or liver cytosol or sulfhydryl compounds (113).

Studies with fly ash suggest that mutagens in the ash may be extracted by serum and that the mutagens are solubilized by protein binding (50).

From our present knowledge, it is clear that lung constituents interact with the mutagens associated with particles in an immediate and substantial manner. Unfortunately, with the exception of the macrophages, we have very limited knowledge of the fate of the organic compounds associated with particles as regards other cells or tissue structures within the respiratory tract. Such knowledge would be useful in interpreting the results of the various in vitro assays in which the target cells are exposed directly to the compounds of interest. Histologically the location of the particles can be observed at various times following exposure. It is not known if any organic compounds remain associated with particles for long periods of time, but such a possibility cannot be excluded.

In addition to information on the fate of inhaled materials within the respiratory tract, it would be useful to have knowledge of the potential translocation of the particle associated organic compounds or trace elements from lungs to other tissues. Unfortunately, only limited data are available on a few specific compounds administered at high levels compared to the concentrations found in ambient air. Studies with BaP and 1-nitropyrene indicate they
are rapidly translocated via the blood to other tissues such as liver and kidney and that urinary excretion occurs (108,109). Additional studies are needed with other compounds, and it is also necessary to more adequately quantitate the clearance patterns of both the parent compounds and their metabolites.

**Local Effects of Inhaled Materials**

In studies completed to date, all of the observed health effects of inhalation exposure to automotive emissions or materials collected from power plants have been nonneoplastic in nature. In general, the responses have been similar in all laboratory animals. The most substantial information has been obtained on animals exposed to diesel exhaust (102,114,115). This information has been reviewed and will be summarized here.

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 histocytes, there is no evidence that other surrounding cells are affected by their presence.

Samples of particulates from coal and oil combustion in boilers and power plants have also been investigated. In inhalation studies of coal fly ash, no significant biological effects were found in a number of animal species (50). Effects of coal and oil combustion particles have been reported in rabbit or hamster macrophages after intratracheal instillation or in vitro exposure (50).

The responses in lung and lymph nodes observed to date represent the usual response of lung to inhaled particles of a relatively insoluble form (8, 102). 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 and physiological changes observed in tissues and airway fluids have in general been transient in nature (6).

**Cancer Induction Studies**

Motor exhaust and coal combustion products have been investigated for carcinogenic activity in whole animal studies by two main experimental approaches. One approach consists of testing single compounds identified as present in these products; the other approach is based on carcinogenesis studies of animals exposed to the complex emission products as complete mixtures of gaseous and particulate phases or to their fractions or extracts.

Each of these two approaches has some advantages and some disadvantages. Studies on individual compounds are important in providing a baseline of information on the presence of carcinogenic materials in the emissions and on their mechanisms of metabolic activation and biological effects. Such studies, however, cannot provide adequate information on possible interactive effects among the various components of complex mixtures. Studies on the carcinogenic effects of complete emissions, their fractions or extracts, are useful for obtaining information on the types of effects of such complex products and for comparing the biological effects of different samples. The limitations of these studies include those involving the representative nature of the samples, the adequacy of the route and mode of administration in providing target tissue exposure, the adequacy of the sample composition and dose in providing an effective balance of toxic and carcinogenic effects, and the sensitivity of the bioassay system in providing a representative response to the chemical and physical properties of the components of the test sample and their interactions.

The whole animal studies involve administration of the test material by subcutaneous injection, skin application, intratracheal instillation or inhalation exposure. Each of these modes of administration has its advantages and limitations which will be briefly summarized below along with major observations made with each mode.

**Subcutaneous Injection.** The subcutaneous injection method may be used for administering soluble or particulate materials. Conduct of studies with this route of administration does not require specialized facilities or skills, and relatively large
numbers of animals can be studied because the expense per animal is low. For particulate materials or compounds that do not translocate to remote organs, the target cells are in the subcutaneous tissue and give rise to sarcomas. Some materials may be translocated and produce tumors at distant sites, e.g., lung adenomas in mice.

Subcutaneous injection has been used to administer pure PAH, extracts and extract fractions of coal-fired domestic furnaces, condensates, extracts and fractions of car exhaust and extracts and fractions of ambient air. The results of these studies have been reviewed (116). These studies showed that PAHs with four to seven rings induced sarcomas, although there were great differences in the potency with the most potent requiring only a few micrograms to induce sarcomas. The potential for coal furnace emissions or ambient air particulate matter to induce sarcomas was fairly well related to the content of PAH, and particularly BaP in the samples. The sarcoma-inducing activity of gasoline engine exhaust condensate was rather low, and, in fact, the effect of BaP was reduced by adding exhaust condensate to the injection sample (116).

**Skin Application.** Skin application of test material has served as a convenient approach to developing information on the two-stage model of carcinogenesis. In this system, skin tumors can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation phase) followed by repetitive treatment with a non-carcinogenic tumor promoter (promotion phase). This system can not only be used to assess the tumor initiating and promoting activities of materials, but by repeated administration of the test material, it also will determine if the material is a complete carcinogen, i.e., if it has both tumor-initiating and promoting activities. The system may involve concurrent administration or agents to determine if they act in an additive, synergistic or antagonISTIC manner with regard to initiation or promotion of tumors or cancer. Major advantages of the system are the moderate manpower requirements and the ease of studying statistically meaningful numbers of animals, thereby allowing a relatively large number of materials to be evaluated. These advantages stem largely from the convenience of applying materials to the skin and directly visualizing effects. The data from skin studies must be extrapolated to other tissues, lung for example, with caution. In addition, the system is most responsive when the test material can be applied in a soluble form. The usefulness of the mouse skin system for studying airborne emissions has been reviewed (117).

Extensive studies have been conducted with SENCAR mice to assess the tumorigenic and carcinogenic activities of organic extracts from a variety of particulate emission sources: diesel and gasoline vehicles, coke oven and roofing tar (117,118). The SENCAR mouse strain was developed by genetically selecting for sensitivity in the 7,12-dimethylbenz(a)anthracene/12-0-tetradecanoyl-phorbol-13-acetate two-stage carcinogenesis model. The data clearly illustrate the tumor-initiating capacity of extracts from particulate samples collected from vehicles manufactured by four different firms. The tumor-initiating activity was observed ranging from inactive for material from one industrial diesel engine to highly active for material from one of the diesel automobiles. Tumors were also produced by extracts of particles from a vehicle using non-leaded gasoline and equipped with a catalyst emission control system. Tumor initiation studies with diesel exhaust particle extracts conducted in C3H mice are in progress, and a preliminary analysis indicates the results are negative (119).

The initiating activity of extracts of particles from roofing tar and coke ovens was compared with the most active diesel exhaust sample using SENCAR mouse data. The activity of the different samples is within an order of magnitude on a per unit weight of tested material basis. Tumor promotion studies with the same materials showed the roofing tar and coke oven-derived materials to be effective promoters. Data from complete carcinogenesis experiments after one year of observation indicate that the diesel exhaust particle extracts are negative as complete carcinogens. In contrast, the coke oven and roofing tar origin samples, as well as BaP, are all positive, with carcinoma incidences ranging from low for the roofing tar to very high for the BaP and coke oven material. Consideration of the BaP content of the complex mixtures indicates that even for the most active diesel exhaust sample, no more than 30% of the papilloma response could be attributed to the BaP alone.

Quantitative analysis of tumor incidence data obtained using SENCAR mice and a log probit model with background correction suggested the following ranking: BaP > coke oven mains > topside coke oven > the most active diesel exhaust sample = roofing tar. Applying a nonlinear Poisson model with background correction for the tumor multiplicity resulted in the following ranking: topside coke oven > most active diesel sample > roofing tar > intermediate diesel exhaust sample = gasoline exhaust sample (117,118).

**Intratracheal Instillation.** The intratracheal instillation method is suitable for administering soluble or suspended materials directly to the lung, which is one of the prime target organs of interest. It does not exactly mimic inhalation exposure because
the nasopharyngeal and tracheobronchial regions are bypassed during the administration process. Some tracheobronchial exposure occurs because a portion of the instilled material is cleared via mucociliary activity. There are also differences in the distribution of the administered material between instillation and inhalation exposures (120). The technique is relatively easy to use since it does not require unique facilities. As a result, it has been used in a number of laboratories with a wide range of materials.

BaP has been the compound most extensively studied with intratracheal instillation (121). It has been administered as the pure compound and on a number of different kinds of carrier particles. The physical characteristics of the carcinogen-particulate preparations were found to be important in determining the retention of the carcinogen in the lung and the level of carcinogenic response. The particle size of the carrier dust, the particulate state of the carcinogen itself, and its physical relationship to the surface of the carrier particles have all been identified as important factors in pulmonary carcinogenesis. Using the appropriate methods, it was possible to produce a high level of carcinogenic response from the respiratory tract epithelium of Syrian hamsters or rats of a type closely comparable to human bronchiogenic carcinoma (122,123).

Only limited work has been done with intratracheal administration of complex mixtures of materials, for example, extracts from urban air particulate samples (116) and condensates from gasoline exhaust (124). The results of the study of urban particulate gave a higher tumor incidence rate than would be expected for pure BaP. This indicates that a mixture of PAH extracted from airborne particles may cause an enhanced carcinogenic effect in the lung in comparison to the content of BaP alone (116). Intratracheal instillation studies with extracts of diesel exhaust particles have been initiated by use of Syrian hamsters (125). Similar studies with emissions from burning of coal and oil have been initiated in Sweden (59). Additional studies of this type will be useful in understanding the carcinogenicity of complex mixtures and may provide a basis for subsequent studies using inhalation exposure.

Inhalation Exposure. Inhalation exposure techniques may be used with gases, particles or combinations of the two. This includes the exposure of animals to complex mixtures of material such as whole vehicle exhaust. A major advantage of this mode of exposure is that it mimics the manner in which the respiratory tracts of people are exposed. A major difficulty is the need for highly specialized facilities and a scientific team that includes aerosol scientists as well as biomedical personnel. The cost of such studies preclude their routine use and requires that inhalation exposure studies only be done when there is evidence for a potential respiratory carcinogenic response from other tests or because concern for inhalation exposure to the material is overwhelming.

With regard to the issue at hand, three types of inhalation studies have been conducted; with BaP alone or in combination with other agents, with vehicle exhaust and with vehicle exhaust in which the animals were pretreated with a known carcinogen. BaP exposure of Syrian hamsters at high levels (total average dose of over 100 mg/animal) resulted in a high incidence of tumors of the larynx, nasal cavity and trachea (126). The lungs were free of neoplastic growth. When rats were exposed to high concentrations of BaP and the irritant, sulfur dioxide, an increased incidence of squamous cell carcinomas of the lung were observed (123,127). Syrian hamsters that were exposed in a similar manner had no significant pathology.

A few studies have been conducted with animals exposed to exhaust (Table 2). In addition to those listed, studies will be initiated during the next year in Japan by the Japan Automobile Research Institute, in Switzerland by the Battelle Geneva Laboratories and in West Germany by the Fraunhofer Institute for Toxicology and Aerosol Science. In considering the studies listed in Table 2, it is important to note that only a few of the studies involve life span exposure and observation. Recognition that if cancers are induced they are likely to occur in low incidence and late in life, places a special premium on observation of the experimental subjects for their full life span. Furthermore, a number of studies have been conducted in which animals were exposed to whole exhaust from internal combustion engines and evaluations focused on nonneoplastic end points (128). To date, an excess incidence of cancer has not been observed in any of the animals exposed only to vehicle exhaust.

Investigators at the Fraunhofer Institute have recognized the difficulty of detecting small carcinogenic effects and have used a novel approach to attempt to detect such an effect (130). They have pretreated Syrian hamsters with subcutaneous itions of diethyl nitrosamine (DEN) 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 is 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 incre-
mental 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 whole diesel exhaust or particle-free exhaust-exposed animals. However, the animals pretreated with the highest dose of DEN and exposed to either whole or particle-free exhaust showed a significantly increased incidence of papillomas of the larynx and trachea compared to the groups receiving only the proven carcinogen (116,121). Moreover, there was no statistical difference in the increase in incidence between the DEN-treated animals that received whole exhaust compared to DEN-treated animals receiving 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. 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 (129).

**Extrapolation of Carcinogenesis**

**Data to Man**

A number of issues must be addressed in extrapolating carcinogenesis data obtained in laboratory animals to man. These have been reviewed and will be briefly summarized here (17). A major issue relates to differences in exposure levels. For man we are typically concerned with environmental exposure levels producing risks of the order of one case per hundred thousand or less. The effects of such levels are obviously not statistically detectable in animal populations of a size that can be economically studied. The experimentalist would require a statistically significant increase of the cancer incidence in treated animals over controls; even with essentially zero cancer occurrence in the control group this requires something close to 10% cancer incidence in the experimental group. Because the exposure levels and associated risks are so different, the investigator is forced to rely on some form of a mathematical model to characterize the exposure-response relationship and extrapolate to the low exposure level region of interest. Several different forms of models are available, including the no-threshold linear model, tolerance distribution models such as the log-probit logistic and “hit” models based on hypothesized processes of carcinogenesis. Beyond these models relating tumor incidence over time, there are models also available that consider time to tumor occurrence. Unfortunately, the animal data available today on the carcinogenic response of animals exposed to complex combustion products are so meager that it is impossible to rigorously evaluate the appropriateness of the several models. The data on some of the specific constituent compounds, such as the PAH, are probably only marginally adequate, at best, for evaluating the relative utility of the several models.

In addition to the issue of extrapolating from high to low doses, other issues must also be considered in extrapolating from laboratory animals to man. These include: within a given species, the effects on metabolism at different exposure levels

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Table 2. Major long-term studies of the health effects of diesel exhaust completed or in progress.

| Laboratory | Species | Particle concentration, µg/m³ | Life-span study | Completed |
|------------|---------|-------------------------------|-----------------|-----------|
| EPA (129)  | Chinese hamster, mice, rats, cats | 6000-12000 | No | Yes |
| Fraunhofer Institute (130) | Syrian hamsters | 4200 | Yes | Yes |
| General Motors (131) | Rats, guinea pigs | 250, 750, 1500 | No | Yes |
| Southwest Research Institute (132) | Mice, rats | 350, 3500, 7000 | Yes | No |
| Battelle-Northwest (133, 134) | Rats | 8300 | Yes | Yes |
| | Syrian hamsters | 7300 | Yes | Yes |
| | Syrian hamsters, rats, mice | 1:60, 1:120, 1:350 dilution | No | Yes |

*Also, exposures to gaseous emissions only without particles.

Particle concentrations not given.
may alter the relationship between exposure level and the dose to the target tissue; reconciliation of the results of experimental studies using exposure to single agents and the simultaneous multiple agent exposure environment of man; and scaling of data between species.

Conclusions

If sufficient human data are not available, animal bioassay data and the results of complementary metabolic and mechanistic studies can provide estimates of carcinogenic risk to man. The estimates may be qualitative (nonnumerical weight of the evidence), relative (providing linkage to other materials through available human data), or directly quantitative (which must be developed with extreme caution).

The relevance of the studies to predicting respiratory cancer induction increases in the following order: subcutaneous injection, skin carcinogenesis, intratracheal instillation and inhalation exposures. Unfortunately, the cost and duration of the studies increase in about the same order.

Particles resulting from fossil fuel combustion or pyrolysis are not considered to have unique properties influencing their deposition. Therefore their deposition can be predicted from general principles. Likewise, the early clearance of the bulk of the particle mass from the nasopharyngeal and tracheobronchial compartments is similar to that of other relatively insoluble materials. The dose to these compartments is limited by the rapid clearance of the particles and proportional to the concentration and duration of exposure.

In evaluating the fate of the quantities deposited in the pulmonary compartment the chemistry of both the particle matrix and the associated organic compounds and trace elements must be considered. In animal studies at low exposure concentrations diesel exhaust particles are removed from this compartment with half times on the order of a hundred days. At high exposure concentrations with accumulation of large quantities of particles removal is impaired. There is evidence for long-term retention of some trace elements.

In animal studies it has been found that some organic compounds associated with particles rapidly leave the pulmonary compartment. Indirect evidence for this is available from studies of the decrease in mutagenic activity of diesel soot particles contained within macrophages removed from the lung at varying times after exposure to diesel exhaust. Direct evidence for rapid loss of organic compounds associated with particles and their translocation to other tissues is available from the study of synthetic aerosols labelled with radioactive organics.

A slower rate of loss of organic compounds (and bacterial mutagenic activity) from diesel exhaust particles is seen with physiological fluids than with concentrated organic solvents.

Mouse skin carcinogenesis bioassays with tumor sensitive mice were used to study extracts of particles from four diesel cars and one unleaded gasoline car. They indicated a range of carcinogenic activities within one order of magnitude. An extract of particles from one heavy-duty diesel engine was inactive. Coke oven and roofing tar particulate extracts produced carcinogenic activity within the range of the activity of the automotive particulate extracts.

Inhalation exposure studies with some pure compounds identified in combustion emissions have produced neoplasms in the respiratory tract of laboratory animals. In studies completed to date, no increase in respiratory tumors has been observed in laboratory animals exposed to high concentrations of diesel exhaust. Animals pretreated with known carcinogens have shown an increased incidence of respiratory tract tumors following exposure to either whole diesel exhaust or diesel exhaust with particles removed. It is not known if this is a unique promoting property of diesel exhaust or an effect of the irritant gases that are present.

Recommendations

Studies should be conducted with a selected number of representative combustion products and fractions of varying complexity using chemical techniques, short-term bioassays and whole animal assays to provide an improved data base for correlating and interpreting the results of the several different assays. Such studies should include as positive controls some pure compounds identified as human or animal carcinogens. Such correlative studies with pure compounds, fractions and complex mixtures are needed to validate the utility of the short-term tests as predictors of carcinogenesis in the whole animal or man.

Standardized samples of typical combustion or pyrolysis product of fossil fuels are required for use as positive controls in short-term and whole-animal bioassays to aid in comparing the results of studies conducted with different assays and in different laboratories.

Additional information is needed on the long-term retention of combustion product particles in the respiratory tract of non-rodent species with
particle retention characteristics similar to man. Additional information is needed on the disposition in the respiratory tract and other tissues of organic compounds inhaled in association with particles or as gases.

Information is needed on the effects in animal assays of selected combustion products using animals with pre-existing respiratory diseases or receiving concurrent exposure to other materials to assist in determining potential risk to sensitive human subpopulations. Pretreatment of animals with known carcinogens provides a method for increasing the background incidence related to exposure to the test material.

Several types of animal studies may be needed to determine the carcinogenic potential to man of airborne combustion and pyrolysis products because of the complexities of the products and limitations of each type study. Each of the several types of studies described in the text has its advantages and disadvantages including relevance, sensitivity, specificity and cost. The use of subcutaneous injections provides for the assay of both local sarcoma induction and systemic induction of tumors in distant organs by carcinogens that are translocated. The mouse skin assay allows large quantities of extracted materials to be administered to an epithelial target tissue. It can be used to assess initiating and promoting properties as well as complete carcinogenesis. The intratracheal instillation assay allows the direct administration of suspensions and extracts to tissues (tracheobronchial and pulmonary) that are of direct concern for cancer induction in man from airborne materials. The inhalation exposure assays provide exposures that are directly analogous to human exposures to airborne materials. The power of the assay is limited by the quantities that can be deposited and the number of animals that can be studied.

Interpretation of the results of short-term and whole-animal assays requires consideration of the dose delivered to the test system relative to the doses received by target tissues in man under plausible conditions of exposure. This should include consideration of possible species differences in the toxicokinetics of the materials being studied.

**Editorial Comments***

The questions addressed to the symposium participants were answered, although not all of them

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*These comments were written by the editors and were approved by the Editorial Committee after the symposium.
stances, but coal power plant emissions also contain potentially carcinogenic metals, which may not be detectable in common mutagenicity tests.

Suboptimal operating conditions may substantially increase the emission of mutagenic activity both for motor engine emissions and coal and wood combustion emissions.

Although short-term tests may be used for certain comparisons, it was concluded that data from short-term tests alone were not a sufficient basis for regulatory actions, but should be used in combination with data from animal experiments and epidemiological studies. One major reason for this was that many short-term tests measure only genotoxic effects and do not consider epigenetic effects, which may well be of importance for the overall cancer risk.

Studies with skin painting on animals with particle extracts from motor vehicle emissions have demonstrated carcinogenic (initiating) properties for both diesel and unleaded gasoline emissions. Animal inhalation studies using motor exhausts have so far proved negative with regard to lung cancer or other cancers. However, animals pretreated with a known carcinogen demonstrated a higher incidence of tumors upon inhalation of whole or particle-free diesel exhausts. It is not known whether this effect is due to promotion or to some other mechanism.

For several reasons it was concluded that single chemical compounds, like benzo(a)pyrene, were not considered being good indicators of the lung cancer risk.

Considering the extrapolation of cancer risks, the group warned against simple extrapolations from animal experimental conditions to human risk estimates, without consideration of the more complex human exposure panorama which can modify the response. During extrapolation from high doses to low doses, however, a non-threshold linear extrapolation model can be useful in the choice of regulatory strategies for fossil fuel combustion products. An estimate reached by such a calculation should, however, be used with caution.

No final decisions were made in terms of cancer cases associated with exposure to different car exhausts and coal combustion emissions. However, the meeting discussed the need for further research to clarify a number of issues and made several recommendations to that aim.

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APPENDIX

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Meeting on Biological Tests in the Evaluation of Mutagenicity and Carcinogenicity of Air Pollutants with Special Reference to Motor Exhausts and Coal Combustion Products

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