

# Contribution of Organic Particulates to Respiratory Cancer

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This paper presents some of the issues that remain to be resolved in order to assess the risk of cancer related to exposure to organic particulates. Most reviews of the effects of organic particulates from the outdoor environment on the risk of lung cancer show that this source seems to play a minor role. However, as fuel use and chemical composition of air pollutants change, the contribution of outdoor pollution as a cause of cancer may also change. Indoor air pollution is a more important source of exposure to organic particulates than is outdoor exposure. Although there is clear evidence that in occupational settings organic particulates cause human cancer, there has been almost no study of exposure to these types of particulates within indoor settings. Previous research has focused on cigarette smoke as the major indoor pollutant, but more specific characterization of contaminants in both the workplace and the home is required. The health effects of the higher levels of some of these contaminants in the workplace should be evaluated and the results extrapolated to populations exposed to lower levels in the home. Extensive research is needed to characterize organic particulate mixtures appropriately and test them for carcinogenicity. Studies on the health risks of nitropolynuclear aromatic hydrocarbons and polychlorinated dibenzodioxins and dibenzofurans are reviewed, but their contribution to the overall burden of respiratory cancer in humans cannot be estimated at this time. Characterization of mixtures, assessment of exposures, and linkage of exposures to health effects are the objectives of the recommendations proposed for further research.

Attempts to assess human health effects from exposure to organic particulates in air have been conducted for many years. Interest in this topic stems from the recognition that virtually all individuals are exposed to breakdown products of organic materials, including fossil fuels. The general public is exposed in the outdoor environment, in the home and office, and even through their own personal activities. Workers may be exposed to high levels of these materials in their occupational environments. Although the carcinogenic potential of this group of materials has been recognized from early experiments in animals and from epidemiologic studies in working populations, we still are unable to assess the extent of the risk to humans from specific exposures. We still are faced with the problem of characterizing the many mixtures as well as specific organic agents to which humans may be exposed and of trying to measure the health effects from exposure to these materials in the indoor and outdoor environment.

It is inappropriate to attempt a comprehensive review of all the literature that addresses the problems of characterization of organic particulates in the general and working environments of the population and the numerous epidemiologic studies of cancer risks from exposures in workers and urban dwellers. This article, therefore, will attempt to present some of the issues that remain to be resolved in order to assess the risk of cancers in humans in relation to organic particulates exposure.

## Epidemiologic Studies of General Atmospheric Pollution

Most epidemiologic studies that have investigated the relationship between cancer and air pollution have been descriptive in design. Usually, mortality from lung cancer in local areas has been related to measured levels of pollutants in the same area. In general, criticisms of the results of such studies are numerous: problems in the precision of measurement, as well as the relevance of specific air pollutants in complex mixtures, the interrelationship of pollution levels with confounding variables such as socioeconomic status and age, the small number of events that have been related to local pollution, inappropriate methods of data analysis, and lack

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of control of such important confounding variables as occupation and smoking.

In 1976, Shy reviewed the available data on lung cancer and air pollution with special emphasis on the possible role of benzo(a)pyrene (BaP) (1). He reported that the concentration of this substance differs by a factor of 10 between urban and rural areas. The concentration of BaP has been shown to differ in relation to local sources of pollution such as tarring operations. Thus, at specific sites, levels may be 2,000 to 12,000 times higher than the average level reported for the whole city. The review suggested that the level of indoor pollution that results from sidestream cigarette smoke might contribute as large an amount of the total body burden in a year as the ambient urban air. Despite these exposure data suggesting there were differences in environmental pollution between urban and rural areas, the review concluded that, while there appeared to be an excess of lung cancer mortality associated with urban living, this excess was small after appropriate corrections for smoking, and that the evidence for an association of the excess with BaP was limited. Most of the studies reported had not accounted for indoor pollutants and occupational factors that could also play a role in the risk of lung cancer.

A few years later, a task force with worldwide representation reviewed the evidence that lung cancer in urban communities might be related to air pollution (2). The group concluded that the "only factors associated with urban living which are likely to account for much of the urban-rural differences are smoking habits, atmospheric pollution, and in some localities, special hazards associated with particular industries." They also concluded that combustion products of fossil fuels in the ambient air may be responsible for 5 to 10 cases of lung cancer per 100,000 males per year. It should be noted that these reviewers believed use of BaP as an indicator substance or marker of the carcinogenicity of air may not be warranted on the basis of the sparsity of collected data on the levels of that pollutant.

Doll, in a review published shortly after the above report, also indicated that, although cigarette smoking is the most important agent associated with the risk of lung cancer, the effects of the combined agents in general environmental air still may account for about 5 cases per 100,000 persons per year (3).

Speizer recently reviewed the evidence relating lung cancer risks and outdoor air pollution (4). His review again emphasized studies that attempt to explain differences between lung cancer mortality or incidence in urban and rural settings based on variation in air pollution. In addition, an assessment of risks in occupational settings where there are known differences in exposures to fossil fuel products has been included. The author noted that most of the studies are inadequately controlled for the smoking characteristics of the subjects. He concluded that previous estimates on the effects of air pollution were probably maximum figures, particularly since exposure to high levels of products from fossil fuel combustion found in occupational set-

tings do not predict the same high risk of lung cancer as do the dose-response curves derived from community data. Thus, the estimated contribution of air pollution to lung cancer in urban males was accepted as 2% of all lung cancers or 5 cases per 100,000 persons and less than 1% of all cancers.

Since the early 1950s when a substantial proportion of the difference in lung cancer mortality between urban and rural residents was attributed to their exposure to different levels of outdoor air pollution, scientists have gradually lowered their estimates of the number of lung cancer deaths that may be related to ambient pollution. In an analysis of the American Cancer Society's prospective study of lung cancer, men were postulated to have an increased risk of lung cancer from occupational exposures but no increased risk by residential population density; that is, there was not an increased risk from urban versus rural residence. Hammond and Garfinkel came to the conclusion that general air pollution had "very little effect, if any, on the lung cancer death rate" (5).

Most of the papers reviewed have not indicated a specific pollutant in urban air that might contribute to lung cancer with the exception of BaP. Otherwise, pollution has been described by the level of total suspended particulates (TSP). There are, however, several papers suggesting that specific types of local pollution may be associated with increased risks of lung cancer (5-7). Although these studies suffer from some of the same flaws as other descriptive studies of outdoor air pollution, they may warrant further attention because they focus on specific environmental contaminants rather than a general surrogate marker of pollution such as TSP.

Higgins has raised similar issues in his evaluation of current evidence for the risk of lung cancer from air pollution (8). He has analyzed the results of several papers that investigated the effects of exposure to diesel fuels in occupational groups. His conclusion was that, while the early studies showed no carcinogenic effect, later studies did demonstrate a small increase in lung cancer related to exposure to diesel exhausts. For most general populations, increased exposures to diesel emissions are too recent to show a marked effect from exposure to the organic particulates from exhausts. However, there should be continued awareness that there may be future changes in the characteristic composition of air pollution due to changing fuel uses that might warrant concern about projected risks of lung cancer due to air pollution.

The most recent review of the role of air pollution in producing health effects is that of the National Academy of Sciences (NAS) (9). The report notes that, while outdoor pollutants have markedly decreased over the last 20 years, there are still local areas with higher contamination levels and changing patterns both in the type and the dispersion of pollutants. There are also unanswered questions about the interrelationship of outdoor air pollutants and personal risk factors such as smoking and indoor exposures as well as quantification of the

contribution of particulates and other pollutants to the risk of cancer (4,10,11). Such precise information would allow us to assess the risk of cancer based on specific exposures.

In summary, most recent reviews and information regarding the effects of organics and particulates from the outdoor environment on the risk of lung cancer have agreed that currently this source seems to play a minor role in the overall risk of the disease. Smoking is undoubtedly the major factor in the risk. However, as changing needs and technology alter the fuel use and the possible chemical mixtures in the environment, the contribution of air pollution as a cause of cancer may change. Local areas of pollution currently may constitute a source of concern. Unfortunately, studies in the past have not been able to quantify levels of exposure to specific substances. Therefore, we cannot estimate at the present time what might be the contribution of polyaromatic derivatives of combustion products to the overall risk of lung cancer.

## Indoor Pollution

To the extent that organic vapors and particulates play a role in carcinogenesis, indoor pollution may be more of a potential problem than outdoor pollution. A recent review of the problems of indoor pollution has discussed most of the papers relevant to this topic (12). The number of sources of both respirable particles and organic vapors found indoors is larger than in the outside environment. These sources include combustion products, resuspension, heating systems, aerosol sprays, solvents, and pesticides as well as cigarette smoke. In general, the concentrations of by-products from these sources are higher than in the outdoor environment. Exposure of individuals to these indoor pollutants is prolonged, compared to outdoor exposure, thus increasing still further the cumulated dose. The introduction of more energy-efficient building designs as well as different building materials has increased and altered problems in the indoor environment. The emphasis in most discussions about indoor pollution has centered on the impact of smoking, rather than reviewing all possible contaminating sources. There are few studies that have characterized the individual's episodic or integrated exposure to contaminants. It is the individual's dose rather than evaluation of sources of exposure that is important in evaluating health effects.

The Environmental Protection Agency has undertaken a program to measure the exposure of humans to toxic substances from many sources in the environment in order to determine the total exposure of individuals (13). The exposure assessment monitors personal exposure to organics in personal air, outdoor air, drinking water, and exhaled breath. The project entitled Total Exposure Assessment Methodology (TEAM) was begun only a few years ago but already is providing information suggesting that the evaluation of exposures based only on suspected sources may not be adequate. The study focuses on organic volatiles, including chlo-

roform, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene. All or a substantial proportion of these chemicals are received by humans through air, and the range of levels of exposure varies greatly by day of week, time of day, and individual.

Recent expansion of these studies has included an evaluation of larger numbers of subjects selected as part of a probability sample. This study confirms that indoor pollution is a more important source of human exposure to these pollutants than outdoor air (14). Previously unrecognized organic pollutants are often the contaminating substances of indoor pollution. The sources of such contaminants were also unexpected. For example, chloroform in indoor air may result from heating of tap water. Increased benzene levels are associated with smoking.

Many studies have indicated the important role of indoor pollutants in the cumulative exposure of individuals. Both the concentration of potentially hazardous substances and the duration of exposure of individuals are greater for indoor versus outdoor environments. The NAS report suggested that individuals may spend 80% to 90% of each day in home, workplace, automobile, or other confined space (12). Outdoor measurements obviously will not identify these exposures.

Sampling of indoor environments creates additional problems to those of sampling outdoors. Methods of sampling that have been recommended for indoor use include both integrated personal monitoring (13) and instruments to identify peak concentrations (12). However, from the discussion above, it is apparent that characterization of the indoor environment will be difficult because of the wide variation of chemicals and chemical mixtures that may be present indoors. Sources of exposure as well as the levels of exposure vary widely by home and workplace environment. The indoor contamination may be unique to a limited number of homes or workplaces that have a specific set of conditions, such as wood-burning stoves in the home or particular combustion products in the foundry.

Most previous studies have emphasized cigarette smoking as the major source of indoor organics and particulates. While there is no question that this source is very important in homes and offices, it may not be the major indoor source for workplace exposures. Nevertheless, the focus of many reports and studies is still on smoking (12). It is essential that we expand our considerations to include other sources of indoor organic and particulate contamination such as energy sources and consumer products.

Many recommendations regarding research needs in assessing indoor pollution have focused only on the characterization of the contaminants. They have also described only home and office environments and ignored other workplace settings where pollutants are also a source of exposure for individuals, yet an individual receives exposures from several indoor sites, and any risks are a representation of this total integrated exposure. The design of future studies must take this need into account.

## Workplace Exposures

If we wish to evaluate the potential risks from exposure to organics and particulates in the environment, occupationally exposed workers are an important group to study. They receive potentially higher doses of the agent than the general public and thus are more likely to demonstrate health effects from the exposure. The work environment may permit better identification of specific chemical exposures because it is a more controlled environment.

Recently, the expert committees of the International Agency for Research on Cancer (IARC) have evaluated the potential carcinogenic effects of polynuclear aromatic compounds (PACs) (15-17). The first of these volumes examined the question of whether carbon black, mineral oil, and nitroarenes (15) are carcinogenic. Most of the data reviewed evaluated risks in occupational settings. In almost all cases, the studies had no quantification or speciation of the exposing agents. If any assessments of individual exposures were made, they were usually based on recent industrial hygiene samples under current conditions of road-paving operations or roofing, for example. The samples were analyzed either for a nonspecific group of agents such as benzene solubles or total suspended particulates. Industrial exposures to aluminum production, iron and steel founding, coal gasification, coke production, coal-tar products, shale oils, and soots were included for review. In many of these workplace situations, there was evidence of carcinogenic effects from exposure to some phase of the industry or some form or byproduct of the material. However, it was often difficult to determine the exact point or source of exposure. The sites that appeared to be at excess risk of cancer included lung, skin, bladder, and stomach, as well as several other sites.

In many of the monographs, there are statements added to the evaluation of the materials or industry that suggest it is impossible to determine the exact nature of the exposure leading to the excess of cancers. The complex mixtures of PACs produced by the various processes were difficult to characterize and probably differed greatly both by time and place. In most cases, there was no information included on types of exposures. The data that establish the presence of similar mixtures of PACs in the ambient air from different industries are limited. Any testing that has been done is usually recent. The methods of sampling, the sources of samples, and the test procedures have varied by industry, by study and by time, so it is difficult to determine the comparability of exposures. Most of the occupational studies that have identified a cancer risk refer to exposures in the middle of this century or earlier. It is difficult or impossible to know whether these exposures at very high doses can be extrapolated to populations exposed to the general environment in more recent periods. For example, if the nitrated polycyclic aromatic hydrocarbons were to contribute more to the air contamination today than in the past, the mutagenic activity of these agents would make one suspect that

lung cancer rates in the population attributable to this exposure might increase. However, because there is limited information on the characterization of pollutants in the workplace or the environment, it is impossible to extrapolate from workplace exposures to population exposures in regard to the cancer burden related to these agents. We do know, however, that nitrated polycyclic aromatic hydrocarbons have been identified in diesel exhausts and polychlorinated dibenzodioxins, as well as other similar substances, have been identified in fly ash. The potential for exposure is there, but the health effects cannot be predicted.

## Characterization of Organic Particulate Mixtures

The characterization of these complex mixtures is very difficult. Standardization of methods for determining what is in the mixture and how to select substances for evaluation in various test systems or animal models has not been determined. The mixtures are difficult to separate into component chemical parts. What appears to be exactly the same mixture from the same process may demonstrate different chemical mixtures on analysis. Separation into individual chemical components may not be the appropriate method of characterizing these mixtures. The interaction of several of these chemicals in the presence of appropriate conditions may be the factor that results in the cancer. Extensive work is needed in this area to determine how to appropriately characterize these mixtures and test them for carcinogenicity.

The following sections review two groups of substances: nitrated polyaromatic hydrocarbons (PAHs) and polychlorinated dibenzodioxins, dibenzofurans, and related compounds; these groups of compounds illustrate some of the sources of complexity in evaluating health risks from organic particulates. Many of these compounds are highly toxic, mutagenic or carcinogenic in animal systems, but their contribution, if any, to the overall burden of respiratory cancer cannot be estimated at this time.

## Nitropolynuclear Aromatic Hydrocarbons

Nitropolynuclear aromatic hydrocarbons, such as nitroarenes, are increasingly recognized to be widely distributed in the environment (e.g., in fly ash, emissions from incinerators, and diesel engines, etc.) (18-20) and are a class of chemicals that present a risk as genotoxic pollutants. First detected in the environment as components of carbon black phototoners (21), nitrated PAHs may be formed as a result of nitration with O<sub>3</sub>, NO<sub>2</sub>, and peroxyacetyl nitrate in airborne pollutants (22,23) or as a result of a variety of combustion processes (24-26), including the incomplete burning of diesel fuel (27-35). They are not only ubiquitous in the atmosphere but may also be present in high concentrations in confined spaces (e.g., office and home environment) primarily as a result of cigarette smoking (34,36,37). Ni-

troarenes can be present in the atmosphere absorbed onto various particles (e.g., fly ash, diesel emissions, and carbon black), or they may be present in the gaseous phase of sidestream cigarette smoke and automobile exhausts (28,32,36,37). The nitropyrenes appear to predominate among the environmental nitroarenes. For example, 1-nitropyrene and dinitropyrenes account for 20 and 30%, respectively, of the direct-acting mutagenic activity of the particulates from hypothetical diesel exhaust emissions (32,33). It is currently estimated that between 50 and 90% of the mutagenicity of the diesel emissions is due to nitroarenes (38-40). Predictions as to the contribution to the mutagenic and nitroarene burden of the environment have differed widely. While Gibson (41) suggested that diesel emissions will "only add 2 to 4 revertants/m<sup>3</sup>" to air in Los Angeles, Pitts (22) found levels as high as 170 revertants/m<sup>3</sup> at a time prior to significant increases of light-duty diesels. A similar association between nitroarene-induced mutagenicity and traffic intensity in Oslo was reported by Alfheim (42). It should be noted that such high levels of nitroarene-induced mutagenicity occur at a time when diesels account for only 0.5% of the traffic (43). The rate of mutagen emissions by gasoline cars is approximately 1 to 7% that of diesel-powered cars (44-47). Rosenkranz (48) calculated that, assuming the total number of vehicles remains constant and that diesel-powered cars will constitute 10% of the passenger fleet, then the mutagenicity of downtown Los Angeles air, due to motor vehicles, will be 302 to 1224 revertants/m<sup>3</sup>.

Data related to the presence of nitroarenes in cigarette smoke are conflicting. McCoy and Rosenkranz (25) reported that cigarette smoke condensates are readily nitrated to mutagenic substances exhibiting the properties expected of nitroarenes. Additionally, they suggested that nitroarenes also appear to be generated during the smoking of cigarettes enriched with nitrates. The structures of commonly detected nitro PAHs are illustrated in Figure 1, and it should be noted that in each instance it is the kinetically favored isomer that is found.

Although the presence of nitropolynuclear aromatic hydrocarbons has been postulated to occur in tobacco smoke due to the possibility that thermally activated NO<sub>x</sub> could scavenge C,H-radicals, El-Bayoumy et al. (49) reported that the concentration of 1-nitronaphthalene and 1-nitropyrene, if they are present in mainstream smoke, would not exceed 10 ng/cigarette, and the concentration of 6-nitrochrysene would not exceed 1 ng/cigarette. These levels can be compared with those of other tobacco smoke carcinogens such as 4-aminobiphenyl (2 to 4 ng/cigarette), 2-naphthylamine (4 to 27 ng/cigarette), benzo(a)pyrene (10 to 50 ng/cigarette), and 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (120 to 440 ng/cigarette) (50).

Gibson (51) reported that levels of 1-nitropyrene in particulate matter in urban and suburban areas were 0.019 to 0.030 and 0.016 to 0.020 ng/m<sup>3</sup> of air, respectively. In Japan, Tokiwa et al. (23,30) detected a 1-nitro-

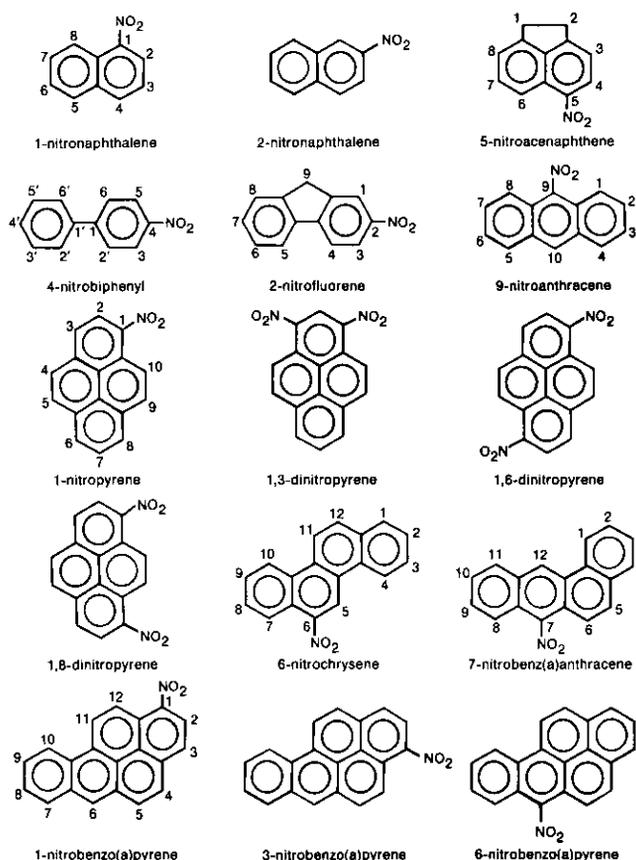


FIGURE 1. Structures of commonly detected nitro PAHs.

pyrene level of 0.21 ng/m<sup>3</sup> in airborne particulate matter. In further studies, particulate matter in an industrial area in Japan was similarly found to contain small amounts of 1-nitropyrene at the levels of 0.072 ng/m<sup>3</sup> in spring, 0.022 in summer, 0.051 in autumn, and 0.045 in winter (52).

Nitropyrenes, as well as other nitroarenes, have been recognized as potent mammalian (20,53) and bacterial mutagens which cause frameshift mutations in *Salmonella* TA 98 and TA 100 in the absence of added microsomes (21,28,30,35,54-56). Part of the concern about the potential health risk associated with nitrated PAHs (e.g., nitroarenes) stems from their "direct-acting" mutagenicity in *S. typhimurium*. Nitropyrenes can be thus considered direct-acting mutagens. It has also been shown that reduction of the nitro function to the corresponding arylhydroxylamine is required for expression of mutagenic potential (20,34,55).

Tester strains deficient in "classical" nitroreductases are less susceptible to mutation induction by 1-nitropyrene (19,54,57,58). The reduction of 1-nitropyrene is accomplished by a bacterial nitroreductase that is distinct from the "classical" nitroreductase that catalyzes the conversion of nitrofurans, nitroimidazoles, and simple nitroarenes (e.g., nitronaphthalenes, nitrofluorenes) to mutagens (54,55,58).

Although 1-nitropyrene has been considered a ques-

tionable "direct-acting" mutagen in mammalian cells (59), it has been shown to induce sister chromatid exchanges in Chinese hamster ovary cells (60,61) and to transform cultured mammalian cells (62,63). Dinitropyrenes (e.g., 1,6- and 1,8-dinitropyrenes) have been shown to be among the most powerful mutagens ever tested (as assayed in *S. typhimurium* TA 98) (19,64).

While the nitroarenes may be the major mutagenic species found in diesel emissions as discussed earlier (28,29,33,48,65-70), it remains to be more rigorously investigated whether these nitrated chemicals are also responsible for the carcinogenicity of diesel emissions (71).

Evidence for the carcinogenicity of nitroarenes is relatively sparse and at times conflicting. The subcutaneous injection of 3-nitrofluoranthene (64,72) and 1-nitropyrene (64,73) resulted in tumors at the site of injection. In addition, 1-nitropyrene caused distinct mammary tumors (73). Sarcomas, mainly malignant fibrous histiocytomas at the site of injection, were induced in 8 of 17 (47%) rats, male (F344/DuCrj), by 1-nitropyrene and in 4 of 10 (40%) rats by 3-nitrofluoranthene. Some tumors were serially transplantable in the same strain of rats. In more recent studies (74), no tumors were found by day 650 in F-344 rats treated subcutaneously with 4 mg and 40 mg doses of 1-nitropyrene. (The previously reported carcinogenicity of 1-nitropyrene (72) was attributed to contamination of the preparation by the dinitropyrenes.) Both 1,6- and 1,8-dinitropyrenes have been found to induce sarcomas at the site of injection in F-344 rats (74). In these studies, 1,6-dinitropyrene was carcinogenic at a total dose of 4 mg and 1,8-dinitropyrene was carcinogenic at total doses of 0.4 and 0.04 mg. 1,6-Dinitropyrene was also shown to be carcinogenic when injected subcutaneously at a total dose of 2 mg into male BALB/c mice, but the injection of the same amount of 1-nitropyrene did not induce tumors within a 420-day observation period (75). Recently, 1-nitropyrene was also reported to enhance the incidence of spontaneous lung tumors when injected intraperitoneally into A/J mice (76). When injected subcutaneously into rats, 1,3-, 1,6-, and 1,8-dinitropyrene induced spindle cell fibrosarcomas. An approximation indicates that these dinitropyrenes were almost as carcinogenic as 3-methylcholanthrene and benzo(a)pyrene (77). Recent studies have also provided evidence that a variety of nitroarenes present in diesel emissions induce *in vitro* oncogenic cell transformations (63,78).

As noted earlier, environmental nitroarenes can be present in the atmosphere, adsorbed onto various particles, and the major route of human absorption of nitroarenes is via inhalation of particulates. As noted by Rosenkranz et al. (36), the ultimate dose of nitroarenes delivered to tissues will depend upon their desorption from the particles and this will differ for fly ash, diesel materials, and carbon black. For example, when 1-nitropyrene is adsorbed onto an inhaled particle, it may be carried up the respiratory tract and swallowed. Upon desorption from the particle, it can be found bound to macromolecules in the kidneys and liver.

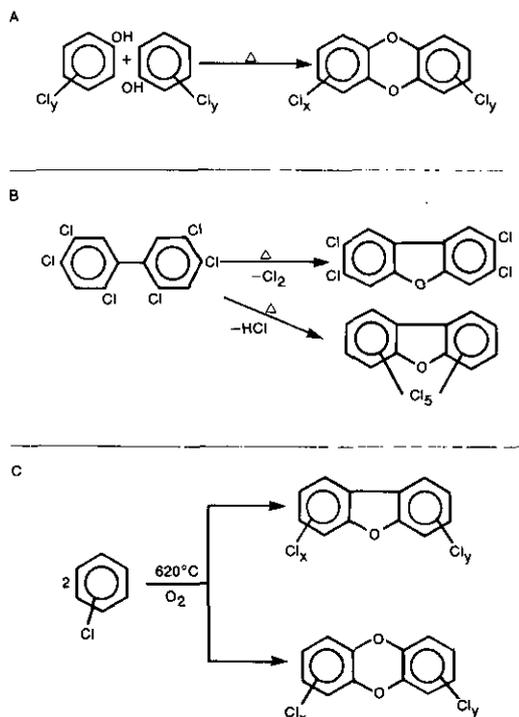


FIGURE 2. (a) Synthesis of polychlorodioxins (PCDs) from chlorophenols. (b) The pyrolysis of polychlorinated biphenyls (PCBs) in the presence of oxygen can lead to the formation of PCDFs. (c) Chlorinated dibenzenes can serve as precursors to the formation of both PCDDs and PCDFs.

## Chlorinated Dibenzodioxins and Dibenzofurans

There is increasing recognition that emissions from urban incinerators, including ashes, fly ash cinders, particulate matter, and organic vapor in the fumes, can contain a large number of chlorinated species, principally polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The PCDDs and PCDFs may be associated with small particulates, which have long residence times in the atmosphere, and in this manner, combustion-generated chlorinated dioxins and furans could become distributed over large areas. Thus, combustion may have made PCDDs and PCDFs ubiquitous in the environment.

Generally, the source of PCDDs are the chlorophenols and their derivatives, whereas the PCDFs are found mostly as contaminants of polychlorinated biphenyls. A typical example of these reactions is the synthesis of polychlorodioxins (PCDs) from chlorophenols (see Fig. 2). The pyrolysis of polychlorinated biphenyls (PCBs) in the presence of oxygen can lead to the formation of PCDFs. Chlorinated dibenzenes can serve as precursors to the formation of both PCDDs and PCDFs.

There are a total of 75 isomers of the PCDDs and 135 of the PCDFs. The latter are generally as toxic as the corresponding PCDDs.

It should be noted that PCDDs and PCDFs have been identified in polychlorinated biphenyl (PCB) samples be-

Table 1. Concentrations (on dry weight basis) of PCDDs and PCDFs in fly ash in several countries.

Compound	Concentrations of PCDDs and PCDFs, ng/g			
	Netherlands	Canada	N. America	Japan
D <sub>2</sub> DD	—	—	—	0.4–200
T <sub>3</sub> DD	—	—	—	1.1–8.2
T <sub>4</sub> CDD	5–110	3.2–27	2.4–85	7.0–250
P <sub>5</sub> CDD	31–490	3.4–45	6.6–210	0.5–310
H <sub>6</sub> CDD	80–1200	2.2–53	9.7–350	0.2–110
H <sub>7</sub> CDD	190–900	1.1–43	5.7–184	Tr–410 <sup>a</sup>
O <sub>8</sub> CDD	110–270	0.4–26	2.1–35	ND–11 <sup>a</sup>
D <sub>w</sub> CDF	—	—	—	ND–20
T <sub>3</sub> CDF	—	—	—	0.7–32
T <sub>4</sub> CDF	13–220	—	4.4–210	0.4–31
P <sub>5</sub> CDF	42–510	—	18–550	ND–180
H <sub>6</sub> CDF	110–870	—	22–1100	Tr–56
H <sub>7</sub> CDF	89–410	—	11–500	0.1–11
O <sub>8</sub> CDF	18–26	—	0.7–27	ND–23

<sup>a</sup>Tr = trace; ND = not detected.

fore they were incinerated (79–81), suggesting that they may be found in the emissions from a poorly designed incinerator, presumably because residence time, oxygen supply, turbulence, atomization, or some combination of all these factors in the reactor zone was inadequate (82).

Since Olie et al. (83) first reported on the occurrence of PCDDs and PCDFs in fly ash from municipal incinerators in the Netherlands, intensive studies have been conducted in Europe, North America, and Japan (84–95). The mechanism(s) of formation of dioxins and dibenzofurans during such processes are very intricate and not fully clarified (96,97).

Polychlorodibenzodioxins and polychlorodibenzofurans, which are usually present in emissions of urban incinerators, yield very similar gas chromatographic profiles, e.g., the species with a higher chlorine content predominate, octachlorodibenzodioxins being the more abundant. Total PCDDs and PCDFs are found in the range of 0.1 to 2 ppm, with 2,3,7,8-tetrachlorodibenzodioxin (TCDD) found at the parts per billion level. For example, TCDD has been found in the fly ash of nine Dutch municipal incinerators at levels ranging from 13 to 373 ppb, while levels of hexachlorodibenzodioxin and octachlorodibenzodioxin ranged from 140 to 1340 ppb and 40 to 1370 ppb, respectively (93).

Studies from nine municipal incinerators in Japan showed PCDDs in fly ash and cinders to be in the ranges of 22 to 870 ng/g and 0.9 to 260 ng/g on a dry weight basis. In most cases the tetrachloro- and pentachlorocongeners were major components in the PCDDs. The concentration levels of PCDFs were nearly the same as those of PCDDs in both fly ash (4.5 to 310 ng/g on dry weight basis) and cinders (1.8 to 74 ng/g on dry weight basis) and were found to be higher in fly ash than in cinders. The concentration ranges of PCDDs and PCDFs in this study (95) are generally at the same levels as those from Europe and North America (Table 1). However, detailed congener comparisons of PCDDs and PCDFs in the Japanese study showed differences (e.g., larger percentages of lower di- and trichlorinated

dibenzo-*p*-dioxins and dibenzofurans and no trace levels of octachlorodibenzo-*p*-dioxin and octachlorodibenzofuran). The differences possibly could be due to higher incinerator temperatures (800–900°C) employed in Japan. Concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in Japanese fly ash and cinders were fairly analogous to those found in Europe and North America. The highest concentrations in fly ash and cinders were 8.0 and 6.7 ng/g for 2,3,7,8-TCDD and 1.3 ng/g for 2,3,7,8-TCDF, respectively, on a dry weight basis (95).

The finding of PCDDs and PCDFs in cinders in the above Japanese study is significant in that the volumes of the cinders are much larger than those of fly ash in municipal incinerators (e.g., cinders amounted to 15 to 30% of the original wastes as wet weight). Consequently, the largest portion of PCDDs and PCDFs in incineration processes is contained in the cinders, not in fly ash. In Japan, disposal of these cinders has been primarily at landfills, raising concern about the fate of these agents in the dumpsite and their subsequent environmental and human impact (95).

As noted above, fly ash extracts and the extracts of particulate matter from five gases contain a complex mixture of PCDDs, PCDFs, and chlorobenzenes. The complexity of the mixtures makes the quantification of the individual compounds very difficult. It should be noted that concentrations of chlorinated compounds vary between different incinerators, as well as between samples taken at different times (93).

The studies of Olie et al. (93) of different incinerators in the Netherlands suggest that while the absolute amounts of the chlorinated compounds vary considerably, the relative amounts (e.g., the proportions of compounds to each other) are fairly constant. Pentachlorobenzene is always the most abundant chlorinated benzene and the hexachlorinated dioxins are nearly always the most abundant dioxins with heptachlorodioxin also being abundant. The relative amounts of chlorinated benzenes are higher in the flue gases than in the fly ash. This may result from the fact that the chlorinated benzenes are more volatile and hence less effectively condensed at the spot where the particles are trapped in the electrostatic precipitator. The above studies of Olie et al. (93) further demonstrated that there is nearly always a good correlation between these three classes of compounds, giving support to the hypothesis that all the compounds are formed from one or several compounds that are in equilibrium with each other. The precursors of these substances are not necessarily compounds that are components of the burned wastes. Table 2 lists the average concentration and estimated yearly output of PCDDs and PCDFs in fly ash and flue gas from municipal incinerators in the Netherlands. The estimated environmental impact of PCDDs from incinerators in Canada and the United States was 13.5 kg/year (98) and 1000 kg/year (99).

The toxicity of the PCDDs and PCDFs in incinerator effluents depends not only on the amount of 2,3,7,8-TCDD but also on the presence of other PCDD and PCDF isomers and congeners, for many of which the

Table 2. Average concentration and estimated yearly output of PCDDs and PCDFs in fly ash and flue gas from municipal incinerators in the Netherlands.

Compound	Fly ash		Flue gas	
	Average Concn, ng/g	Total amount/yr, kg	Average Concn, ng/m <sup>3</sup>	Total amount/yr, kg
TCDD	93	5.6	57	0.8
P <sub>5</sub> CDD	254	15.2	244	3.4
H <sub>6</sub> CDD	604	36.2	440	6.2
H <sub>7</sub> CDD	760	45.6	347	4.9
OCDD	345	20.7	452	6.3
	2056	123.3	1540	21.6
TCDF	173	10.4	161	2.3
P <sub>5</sub> CDF	312	18.7	272	3.8
H <sub>6</sub> CDF	459	27.5	528	7.4
H <sub>7</sub> CDF	314	18.8	293	4.1
OCDF	51	3.1	68	1.0
	1309	78.5	1322	18.6

toxicity has not been rigorously investigated. Table 3 lists the number of toxic compounds per isomer group. The toxicity of only 9 of the PCDDs and 8 of the 39 probably toxic PCDFs has been studied.

Although 2,3,7,8-TCDD has been studied more extensively than other PCDDs and PCDFs and is the most toxic of these compounds, it is generally recognized that it is rarely encountered by itself in the environment and as noted above, it is not always the major contaminant of a PCDD/PCDF mixture. Tiernan et al. (100) found 2,3,7,8-TCDD to be a very minor component of the tetrachlorinated dibenzofurans (about 1% of the total TCDDs) identified in the extract of particulates present in flue gas effluents collected from municipal refuse incineration in Virginia.

Olie et al. (93) suggested that in evaluating the toxicity of incinerator effluents, a factor of 1 for 2,3,7,8-TCDD and a factor of 0.1 for the other compounds with three or four chlorine atoms in the lateral position and at least one hydrogen atom in the other positions should be used. Another assumption that was postulated was that isomers are equally distributed in each isomer group and knowing that 2,3,7,8-TCDD is about 3.3% of the tetra isomers, the toxic equivalent of the extracts relative to 2,3,7,8-TCDD can be calculated. The toxicity of the fly ash extracts from Dutch municipal incinerators was estimated to be about 50 times as high as that based

Table 3. Toxic compounds of PCDDs and PCDFs per isomer group.

Compound	Total number of isomers	Number of toxic isomers	Toxic isomers, % of total
TCDD	22	4 (+2,3,7,8)	18
P <sub>5</sub> CDD	14	7	50
H <sub>6</sub> CDD	10	7	70
H <sub>7</sub> CDD	2	1	50
TCDF	38	8	21
P <sub>5</sub> CDF	28	14	50
H <sub>6</sub> CDF	16	12	75
H <sub>7</sub> CDF	4	2	50

on 2,3,7,8-TCDD and for the flue gas the factor was about 80. Olie et al. (93) concluded that the hexa- and heptachlorodioxins and hexadibenzofurans contributed largely to the toxicity of the extracts while it was unlikely that 2,3,7,8-TCDD contributed most of the toxicity of the extracts.

The preponderance of toxicity studies involving the polychlorinated dibenzodioxins has focused on TCDD since human exposure to TCDD has resulted from industrial accidents, uncontained chemical wastes (e.g., Love Canal, Times Beach episodes) and the use of the defoliant Agent Orange. Studies conducted in animals indicate that TCDD and its isosteric analogs act through a common receptor to produce a characteristic pattern of toxic responses (101-104), which include a slow wasting syndrome, teratogenesis, and thymic atrophy. Hyperkeratosis and chloracne are observed in a limited number of animal species but are among the most widespread responses observed in humans.

TCDD is a potent inducer of many proteins including drug metabolizing enzymes such as the cytochrome P-450 proteins (e.g., P<sub>1</sub>-450 and P<sub>3</sub>-450). Induction of P<sub>1</sub>-450 by TCDD may play a role in the initiation of certain types of environmentally caused malignancies.

Treatment of the human breast carcinoma cell line MCF-7 with TCDD has recently been shown to result in high levels of arylhydrocarbons, i.e., benzo(a)pyrene, hydroxylase (P<sub>1</sub>-450) activity. This cell line was used to isolate a human P<sub>1</sub>-450 full-length complementary DNA (cDNA clone) (105).

TCDD is a cocarcinogen in a mouse tumor model system and is a very potent promoter of tumorigenesis. Chronic exposure to low concentrations of TCDD is carcinogenic at multiple tissue sites in both rats and mice (106-108). Although TCDD is a potent carcinogen, there are conflicting reports concerning its genotoxic potential. Early studies for which little experimental data were provided suggested that TCDD was a direct-acting mutagen for *Salmonella typhimurium* (109,110) and *E. coli* (110). More recent studies have failed to confirm these early bacterial mutagenicity studies (111,112). Additionally, negligible levels of covalent binding of TCDD to biological macromolecules have been observed *in vivo* and *in vitro* (103,104,113,114). Recent studies have also shown that TCDD is a potent liver tumor promoter in the rat (115) and that it can promote skin tumor formation in the HRS/J mouse (101,102). TCDD has also recently been shown to promote the transformation of C3H/10T1/2 mouse embryo fibroblasts (116). Taken together, these findings suggest that the carcinogenic effects of TCDD are not mediated by direct genotoxic events. The evidence for the carcinogenicity of TCDD in humans is equivocal (117,118).

In a 2-year study (106), rats were fed diets containing TCDD at three levels (0.001, 0.01, and 0.1 µg/kg body weight/day). The highest level led to hepatocellular carcinomas and squamous cell carcinomas of the lung. A dry diet was used which may have permitted inhalation of particles with consequent TCDD exposure of the lung.

As evident from the preceding discussion, organic particulate mixtures are characterized by a multiplicity of component compounds which have demonstrated mutagenic or carcinogenic properties in standard test systems. Furthermore, a great many other related compounds, which have not been discussed, are present in such mixtures. Clearly, testing for all possible compounds from both indoor and outdoor environmental exposures is not feasible. Therefore, it is necessary to choose an identifiable set of "marker or surrogate agents" collected under standardized conditions for which testing may be readily accomplished. Once this set of "marker agents" has been selected, the second step involves determining how the presence of these "markers" relates to the potential for human disease resulting from exposure to these and other agents in the environment.

Thus, the solution of this problem involves a two-step process. First, the universe of possible test compounds must be limited to a selected few in order to make testing feasible. Second, once the markers have been selected, a method must be developed to relate the presence of such markers to the potential for human disease. These procedures are clearly complex and involved, and their solution will require an integrated team effort of many scientists and many disciplines.

## Research Recommendations

All humans are exposed in some ways to fossil fuels and organic particles, and exposures will probably continue for many years. In the past, emphasis has been on general measurements of exposure that were not carefully characterized in terms of specific human exposure. Assessments have been done of generic exposures to the environment by generic populations. Laboratory studies, on the other hand, have emphasized the mutagenic and carcinogenic effects of selected specific compounds from among the many that are present in the environment.

There is clear evidence that in occupational settings organic particulates cause human cancer. There has been almost no study of the exposure of individuals within indoor settings to these types of particulates or vapors.

To a large extent, risk assessment of organic particles must follow the principles of risk assessment for chemical mixtures. This is a relatively new field, at an early stage of development. Guidelines are being developed, but at present each risk assessment of a chemical mixture must be handled on an individual basis. Some mixtures, such as diesel exhaust, have been relatively well characterized in terms of chemical composition, particle size distribution, and biological effects. Many other chemical mixtures are not well characterized, and risk assessment cannot be done unless it is established that the mixture is "like diesel exhaust," or that the toxicity of the mixture can be characterized by the activity of one or more surrogate components of the mixture. Characterization of mixtures, assessment of exposures, and

linkage of exposures to health effects are the objectives of the following recommendations.

## Characterization of Chemical Mixtures

**Recommendation 1:** Standard laboratory procedures should be developed to identify the components of organic particulate mixtures and to determine their stability and biological activity.

Because of the importance of indoor pollution and the paucity of studies of this environment, it is recommended that a standard indoor mixture sample be added to the current International Collaborative Study for Assessment of Complex Mixtures. In this way, an effort could be made to standardize indoor as well as outdoor pollutants.

**Recommendation 2:** Primary standards should be developed for comparison with other mixtures. This would enable the assessment of the relative potency of materials in the same classes of mixtures through standardized procedures.

**Recommendation 3:** Surrogate chemicals should be selected that may be markers of mixtures. Surrogate chemicals could perhaps be suggested for specific kinds of markers of groups or classes of mixtures. These could be developed for laboratory standards.

**Recommendation 4:** Mixtures should be selected for study based on their relevance to known human exposures in outdoor, indoor, and occupational environments. Laboratory studies should be done of mixtures identified in actual human exposure situations. This will require collaboration between toxicologists and other laboratory scientists.

**Recommendation 5:** Guidelines and procedures should be developed to predict the biological activity of specific mixtures based on the structure of the components of the mixture. This is a difficult problem to address, because the activity of mixtures may be very different from the activity of single chemicals.

**Recommendation 6:** A strategy should be developed to select chemicals and/or mixtures for further study that would be the important markers for relevant biological endpoints. This applies primarily to the chemical characterization of mixtures.

## Animal Studies

**Recommendation 7:** Animal models should be developed that can be used to titrate exposures and to measure biological endpoints relevant to the assessment of human exposures.

Such animal models could be used to determine what biologic fluids should be sampled and for what periods, whether peak exposures or cumulative exposures are more important for given biologic endpoints, and other issues. Such determinations would involve toxicokinetics of single chemicals and interactions of chemical mixtures, as well as interspecies differences in xenobiotic metabolism.

## Human Data

**Recommendation 8:** There should be continued study of occupational groups exposed to fossil fuel products to obtain dose-response information that can be used to extrapolate to low dose-exposed populations.

Environmental measurements need to be developed using appropriate surrogate markers for the mixtures that are relevant to effects in animals. Such surrogate measurements of the mixture can be applied to biological endpoints in animals and then applied to humans. The surrogates may differ for different mixtures, but a single surrogate may be found for classes of mixtures that can be used in human populations, particularly in the many occupationally exposed groups.

**Recommendation 9:** Opportunities to study other occupational groups that may be used for similar epidemiologic purposes should be explored.

**Recommendation 10:** Biological monitoring systems should be developed to determine exposures and their relationships to potential changes in humans.

**Recommendation 11:** Personal monitoring devices should be developed that could characterize particulate samples from an individual's breathing zone. This would provide the means of integrating the individual's exposure throughout the entire day in the home, at work, etc. An integrated dose to the individual over a period of time could be determined.

## Development of a Model of Human Exposure to Indoor Air Pollution

**Recommendation 12:** A model of human exposure to indoor air pollution needs to be developed. The development of such a model should include a sampling strategy to determine which homes should be sampled; a method to set priorities for source strength emission studies of building materials, consumer products, and other point sources of exposure within homes; and a pilot survey to determine whether the model is measuring human exposure.

The development of a sampling strategy to identify homes that could be studied to characterize the exposure of the U.S. population is a difficult problem. However, a strategy has been developed for the study of radon.

The next step in the development of a model would require data to characterize homes. Information would be needed on the point sources and source strength of emissions. Because of the large numbers of potential sources of pollutants such as building materials and consumer products, it will be necessary to identify the most important sources for study so that not every home will have to be sampled.

With the addition of human data that have been collected from homes and by personal monitors, it should be possible to model the kind of exposures humans might experience in the indoor environment. The validation of the model will determine whether the model is relevant to actual human exposures in the home.

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