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Bioavailability and Biotransformation of the Mutagenic Component of Particulate Emissions Present in Motor Exhaust Samples

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The pharmacokinetic concepts of bioavailability and biotransformation are introduced into the assessment of public health risk from experimental data concerning the emissions of potentially mutagenic and carcinogenic substances from motor vehicles.

The inappropriateness of an automatic application in the risk assessment process of analytical or experimental results, obtained with extracts and procedures incompatible with the biological environment, is illustrated on the discrepancy between short-term laboratory tests predictions that wider use of diesel engines on our roads will increase the risk of respiratory cancer and the widely negative epidemiological evidence. Mutagenic activity of diesel particulates was minimal or negative when tested in extracts obtained with biological fluids, was substantially dependent on the presence of nitroreductase in the microbial tester strain, and disappeared completely 48 hr after the diesel particles had been phagocytized by alveolar macrophages. Similarly, long-term animal inhalation exposures to high concentrations of diesel particles did not induce the activity of hydrocarbon metabolizing enzymes or specific adverse immune response unless organic solvent extracts of diesel particles were administered intratracheally or parenterally in doses that highly exceed the predicted levels of public exposure even by the year 2000. Furthermore, the suspected cancer producing effects of inhaled diesel particles have thus far not been verified by experimental animal models or available long-term epidemiological observations.

It is concluded that unless the biological accessibility of the active component on the pollutant as well as its biotransformation and clearance by natural defense mechanisms are considered, lung cancer risk assessment based solely on laboratory microbial tests will remain an arbitrary and unrealistic process and will not provide meaningful information on the potential health hazard of a pollutant.

Since the time when Ehrlich (*1*) identified hypothetical chemical ligands in the cell interior (receptors) on which chemicals entering the living organism act, it has been believed that whatever the effect of a chemical in the biological system is, it occurs as a consequence of physicochemical interactions between the chemical and some functionally important chemical structure in the system. This obviously implies that the possibility of the drug reaching the receptor in satisfactory concentration is a necessary prerequisite for a measurable biological response. Recently, modern pharmacology (*2*) has been testing clinical efficacy and therapeutic potential of new drugs by measuring the drug transfer from the site of administration into the general circulation. Similarly, environmental toxicology must realize today that for proper understanding of the ultimate effects of environmental pollutants, it cannot depend only on measurement of the applied dose (exposure), but must also determine what is the possibility and velocity with which the environmental pollutant or its effective component can reach the target organ, cell, or specific chemical structure. In fact, the most effective assessment of the response would be to measure the concentration of the chemical compound directly at the receptor site. This is still an idealistic approach even in pharmacotherapy and today's

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pharmacology estimates the bioavailability of the drug primarily from the change of the drug concentration in the circulating plasma with time.

Naturally, solubility of the administered form of the drug in the biological environment, i.e., extracellular fluid and serum or plasma, is of cardinal importance for the drug distribution via systemic circulation and, at the same time, it is an easily measurable parameter in laboratory conditions. Indeed, solubility in biological fluids is a basic prerequisite for the manifestation of any biological effect; should the drug be administered in the insoluble form, no drug will reach the target organ receptors, and the expected pharmacological response will not occur.

When identical principles are applied to environmental toxicology, a direct parallel in the need for a more exact quantification of the adverse response to the pollutant can be easily recognized. The effects of an environmental pollutant are also determined by its possibility to reach its target in the organism. In pharmacology, the form of drug administration is preselected not to interfere with the integrity of tissues at the site of injection. In contrast, the entry of an environmental pollutant occurs via variable routes, and a strong possibility exists for an undesirable effect at the site where the pollutant enters into the organism. Indeed, in many cases, the local effect is the dominant action of the pollutant. In other cases, the inhalation of aerosols may result in a subsequent retention of the particulate matter in the respiratory system and formation of a permanent depot from which the active component is continually distributed to a distant receptor or, what is more important, the reactive chemicals may be directly released into sensitive cells of the respiratory system that are in intimate contact with the deposited particle (Fig. 1).

Ultimately, the final effect is not determined only by the absolute size of the dose entering the organism, but also by the port of entry, site of deposition, *in vivo* availability of the active components released and distributed through the organism, and by the final form of the chemical which may be either activated or detoxified by the action of the cellular metabolizing enzymes. Therefore, only a complex evaluation of all factors involved can provide a realistic rationale for the true and meaningful assessment of the real health hazards and population risks.

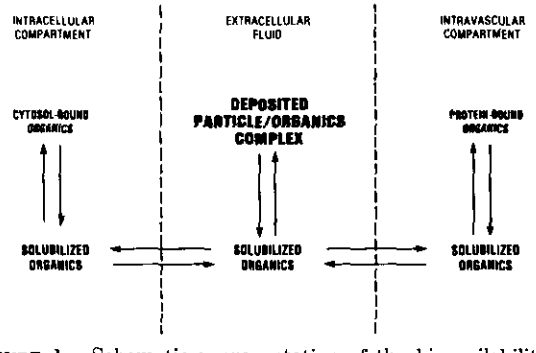

FIGURE 1. Schematic representation of the bioavailability of particulate polycyclic organic matter.

Particulate Polycyclic Organic Matter (PPOM)

Numerous polycyclic hydrocarbons have been identified in urban air, including pyrene, phenanthrene, fluoranthene, benzoperylene, benzo(a)pyrene, benzofluoranthene, chrysene, and arise primarily from combustion of organic matter. However, the presence of polycyclic hydrocarbons in our environment is ubiquitous (*3*), and significant natural emissions of terpenic hydrocarbons by conifers occur continuously in many evergreen forests (*4*). Man-made sources are primarily represented by hydrocarbon emissions from stationary sources and the emission inventory for benzo[a]pyrene indicates the ratio between stationary and mobile sources in the United States to be approximately 30:1 to 50:1(*5*).

The levels of all polycyclic aromatic hydrocarbons in the ambient air and even in tobacco smoke are well below their experimental thresholds for complete mouse skin carcinogenesis (*6*). However, the evidence for the induction of lung cancer by inhaled cancer-causing hydrocarbons is highly suggestive, and many environmental factors, i.e., cigarette smoking and occupational exposures, have been proposed as responsible for the increased hazard of chemically induced neoplasia in the respiratory system.

In spite of the generally accepted interpretation, there has been no direct experimental evidence that inhalation of a specific polycyclic hydrocarbon has caused respiratory neoplastic processes in man, and Kuschner et al. (*7*) reported that exposures to polycyclic hydrocarbons, with defined carcinogenic potencies established in skin-painting tests, do not produce lung cancers in experimental animals, even at extremely high concentrations (10 mg/m^3). The positive proof of carcinogenicity depends primarily on tests in which local tumors were produced by a prolonged administration of excessive doses on the animal's skin. Nettesheim and Griesemer (*8*) and Laskin and Sellakumar (*9*) explained the surprising fact that most of the inhalation studies reported negative results, by deficiencies in aerosol genera-

tion technology and inadequate experimental design. Scala (*10*) emphasized the necessary presence of additional factors which can promote the carcinogenic action of the hydrocarbons; without promotion, carcinogenic potential may remain unmanifested.

Many alternate approaches were developed as an experimental model of respiratory tract carcinogenesis and included direct injections of the carcinogen into lung tissue, intrabronchial and intratracheal application of carcinogens with other irritating agents, or inhalations of carcinogenic hydrocarbons either simultaneously or sequentially associated with other airborne materials (*8*). The thought that for manifestation of the carcinogenic effect, sensitive cells of the respiratory tract must be chronically irritated by another noncarcinogenic factor, or that the contact of the carcinogen with the specific cell must be prolonged and intensive, has been dominant in all proposed experimental designs. Simple breathing of hydrocarbon vapors present in the ambient air was considered ineffective because the concentrations are usually low, are applied randomly to the entire lung surface, and even after penetration into the cell, they are rapidly detoxified or cleared via the perfusing blood.

At environmental temperatures, the polycyclic organic material in the community or workplace air is largely present in the form of physically dispersed condensed aerosol nuclei, and only trace concentrations exist in the true form of vapor. Although it is uncertain whether the polynuclear aromatic hydrocarbons condense out as discrete aerosol droplets or are physically adsorbed on the surface of particles formed during the combustion process, the presence of submicron-sized carbonaceous particles with a large adsorptive surface process is believed to escalate the condensation of hydrocarbons. When the submicron-sized particles are inhaled and retained in the respiratory system, the intimate contact of adsorbed hydrocarbons with the directly adjacent respiratory cell(s) may be prolonged, lead to the penetration of hydrocarbons into the cells, and result in the manifestation of their biological activity.

Consequently, the rate and efficiency of release of the adsorbed hydrocarbons from the associated particulate matter by the action of alveolar or other biological fluids is of crucial importance in predetermining the ultimate biological response and potential adverse health effect of the deposited particles.

Table 1 lists the most important representatives of the particulate polycyclic organic matter to which man can be exposed either in the ambient air or in his occupation and compares the mass fractions of the particles which are extractable organic matter and the concentrations of benzo[a]pyrene in the extract. Unfortunately, the solvent soluble fractions have been obtained using different organics solvents; repeated extraction by toluene was used for carbon black, dichloromethane for diesel and gasoline engine particles and benzene for ambient aerosols and coke oven emission. As a consequence, the total mass as well as the extractable hydrocarbon fraction representation may have changed. Cigarette condensate (*6*, *12*), roofing tar extract (*12*) or automobile exhaust condensate (*17*, *18*) were not included in the list, since they do not comply with the definition of the particulate-organics association. The particulate phase of cigarette smoke consists entirely of liquid aerosol (tar) and is, therefore, 100% soluble in an organic

Table 1. Various types of particulate polycyclic organic matter.

Source	Particle size, μm	Solvent soluble, %	Benzo[a]pyrene concentration μg/mg ext.
Carbon black	0.1 –0.2	0.08–0.13	0.02 –0.05[a]
Diesel particles	0.15–0.2	12–17	0.002–0.026[b]
		10–15	0.09[c]
Gasoline exhaust	0.15–0.2	39–43	0.1[b]
Nonurban aerosol	0.16–0.21	2–8	0.0–0.17[d]
Urban aerosol			
Continental	0.16–0.21	7–13	0.15–0.61[d]
Maritime	—	6–9	0.21–0.26[d]
Coke oven emissions	0.1 –1.0	5–10	0.5[b]
		10	5.0[e]
		10	100.0[f]

[a]Data of Buddingh et al. (*11*).
[b]Data of Huisingh et al. (*12*).
[c]Data of Williams (*13*).
[d]NAS data (*14*).
[e]Data of Jackson et al. (*15*).
[f]Data of Schulte et al. (*16*).

solvent. Depending on the makeup of the cigarette, the condensate represents 0.2-9.0% of the weight of mainstream smoke (500 mg/cigarette). Since the total benzo(a)pyrene content is 10-50 μg/cigarette, this represents a low concentration of 0.01-0.05 μg/mg condensate in the average 1960s commercial cigarette. In comparison, the 2RI Kentucky reference cigarette smoke condensate, generated for the U.S. EPA Diesel Exhaust Research Program (*12*) is the result of sizeable per-cigarette reduction of the noxious constituents in the currently available cigarettes and contains benzo[a]pyrene concentrations which are approximately one hundred times lower: 0.0006 μg/mg condensate (*19*). Similarly as the cigarette condensate, the roofing tar extract and automobile exhaust condensate are particle-free and completely soluble in an organic solvent; their benzo(a)pyrene content is approximately 1 μg/mg (*12*) and 0.2–0.3 μg/mg condensate (*17*), respectively.

Bioavailability

The different character of the soot used (carbon black, atmospheric soot, diesel exhaust particles), variability in the experimental design, and problems with the analytical determination of trace amounts of polycyclic hydrocarbons are responsible for the controversy that exists regarding the ability of biological fluids to extract hydrocarbons from the soot particles *in vitro* (*20*).

The attempts to overcome the low sensitivity of the applied analytical methods by enriching the hydrocarbon fraction with excessive amounts of benzo[a]pyrene (*21*) further complicated the problem. As expected, the authors using soot particles with added hydrocarbons find a variable fraction eluted by serum or other biological tissues (*22-25*), whereas investigations attempting to extract the naturally adsorbed benzo(a)pyrene (*26,27*) reported completely negative results.

Falk (*22*) indicated that human plasma eluted benzo(a)pyrene (BaP) only from particles larger than 100 μm, and Obrikat and Wettig (*28*) reported that large species differences exist in the solubility of benzo[a]pyrene and pyrene between the human and animal serum. The transfer of benzo[a]pyrene between the particles and animal tissues was studied by Creasia et al. (*24*) and recently also by Medda et al. (*25*). Both authors used soot or diesel particles enriched with benzo[a]pyrene and reported an early release of the hydrocarbon into the circulation when particles were small (below 1 μm). In contrast, BaP adsorbed on large particles (15-30 μm) was cleared from the lung tissue at a rate identical to the clearance of carbon particles, and the authors admit that the *in vitro* adsorption process may not have correctly simulated the forces by which benzo[a]pyrene is bound during the combustion process. Nettesheim (*8*) studied the release of BaP from beeswax pellets (100 μg BaP adsorbed to 900 μg of activated charcoal and incorporated into beeswax) *in vitro* and after implantation into tracheal transplants in rats; the release of BaP *in vivo* was approximately 2.8 ± 06% per day at the highest concentrations. When small concentrations were used, initial release was rapid and most of the carcinogen was delivered to the graft in the first two weeks. Inspite of the rapid release, no significant preneoplastic or neoplastic lesions were observed. Similar studies were done with 7,12-di-methylbenzo-(a)anthracene. The release from the beeswax pellet occurred with an exponential rate at high administered concentration and represented approximately 1.7% of the amount remaining in the pellet per day. Again at lower concentrations (< 200 μg DMBA) nearly all carcinogen was released within 1 to 4 weeks. The tracheal transplant model may be a feasible carcinogen delivery system for experimental lung cancer induction, however, it can hardly be considered a representative model of the bioavailability of hydrocarbons from soot particles due to its completely artificial character.

Compared to other types of internal combustion engines, the diesel engine produces approximately 30-100 times greater mass of submicron-sized particles, and therefore most of the studies related to the association of potential carcinogenic effects of automotive emission have concentrated on diesel particles (*29*). The particles are submicron in size (0.15-0.2 μm MMAD) and consist of a carbonaceous core on which variable amounts of hydrocarbons adsorbed. (Fig. 2) The hydrocarbons can be extracted by any organic solvent and can be separated from the solid core.

Chemical analysis reveals (Table 2) that the solid core consists practically of pure carbon; its molecular ratio of hydrogen to carbon is many times lower

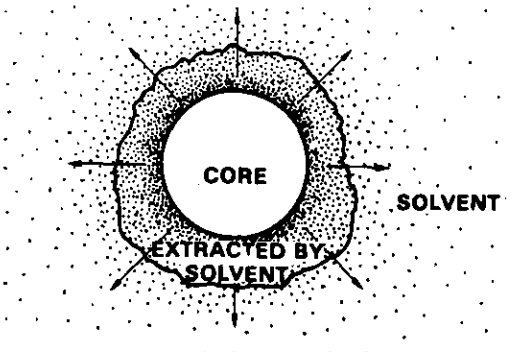

FIGURE 2. Schematic drawing of a diesel particle.

Table 2. Diesel particulate composition.

	Weight-%					
	C	H	O	N	Molecular formula	Molecular weight
Extractable fraction	79.5	10.0	11.3	0.70	$C_{24}H_{39}O_{2.6}N_{0.18}$	150-5000
Dry core	81.9	1.4	16.2	0.60	$C_{6.0}H_{1.2}O_{0.9}N_{0.04}$	

than that of the original fuel and only minimal traces of oxygen and nitrogen are present. In contrast, the hydrocarbon residuum obtained after solvent extraction and careful evaporation of the solvent indicates the presence of compounds with a wide range of molecular weights and a significant hydrogen excess over the carbon and is expected to be composed of highly variable quantities of an estimated 10 to 20,000 hydrocarbons (*29*).

Considering the potential presence of biologically active components that would have serious consequences for human health, chemical analyses indicated first of all that concentrations of benzo[a]pyrene in diesel particulates are much lower than in particulates obtained from precatalyst cars and lower or comparable with those found in particulates from catalyst-equipped gasoline-powered engines (*30*).

Numerous investigators attempted to find a common denominator for the presumed neoplastic action of the polycyclic particulate matter and benzo(a)-pyrene concentrations have been frequently used for a comparative assessment of the exposure risks. Thus Albert (*31*) proposed an intercomparison of the potency of diesel exhaust with the biological activity of coke oven emissions, roofing tar extract, and cigarette smoke condensate. Presumably, the relative activity in animal experimentation or *in vitro* laboratory experiments will be indicative of in a comparable carcinogenic activity in the exposed populations. However, the variability of the benzo-[a]pyrene concentrations, particularly in the coke oven emissions (Table 1), indicates that the coke battery operation has changed significantly during the last decades and that present emissions are not necessarily representative of the material emitted when the exposure of workers occurred (*32*). The potency of samples collected today does not reflect the quality and quantity of the polycyclic organic matter to which the worker's cohorts with increased frequencies of neoplastic processes were exposed 20-30 years ago. In addition, an analysis of the mutagenic activity shows that wide differences between diesel and coke over particulates also exist in the mechanism of their action. Pederson and Siak (*33*) compared the profiles of biological effects of both extracts and concluded that the mutagenic activity detected in bacterial mutagenicity assay of coke oven emission particle extracts requires mammalian liver enzyme activation, whereas the mutagenic activity of diesel exhaust particle extracts does not. Comparisons of the mutagenic activity profiles of the thin layer chromatographic fractions of diesel particle and coke oven extracts indicates that whereas direct-acting mutagenic activity is found in the nitro-substituted hydrocarbon fraction of diesel extract, the activity of coke oven emission particle extracts is found mostly in the polycyclic aromatic hydrocarbons and the polar fractions containing no nitro-substituted compounds (Fig. 3).

However, just as low concentrations of benzo(a)-

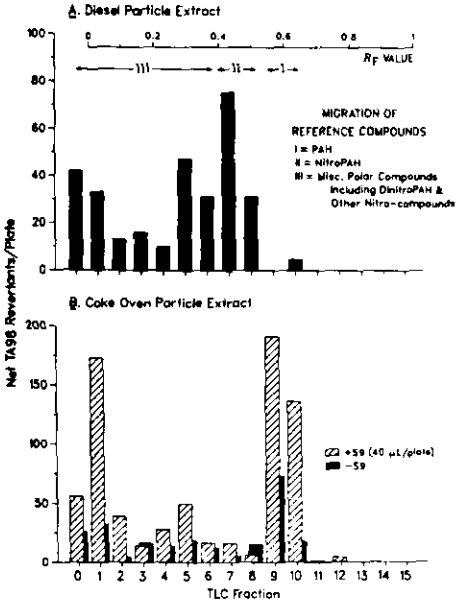

FIGURE 3. Mutagenic activity recovered in TLC fractions from diesel particle extract and coke-oven emission extract chromatographed on normal phase silica gel plate. The fractions were extracted with DMSO and assayed for mutagenic activity in the tester strain TA 98. For diesel particle extract, fractions were assayed without S9, and for coke-oven emission, extract fractions were assayed with and without S9. Areas I, II and III at the top of the chart represent the range of migration for three compound classes as indicated. Data of Pederson and Siak (*33*).

pyrene do not exclude the possibility of the presence of other components with biological activity, the analytical proof itself does not always indicate that the potentially harmful compounds are easily available for the *in vivo* action. Only after the hydrocarbon molecule has left the carbon core of the particle could it cross the cell membrane to interact with the intracellular components and produce a potential error in DNA replication which, if not immediately repaired, can produce genetic mutations or, theoretically, neoplastic conversion of the newly produced cell.

In order to assess their specific biological aggressivity, the hydrocarbons adsorbed on the surface of particulates have been frequently extracted from the particulates and concentrated by using powerful organic solvents which are not present in the living organism. After separation, the biological activity of the extracted hydrocarbons has been tested in laboratory tests, and the resulting biological effects were frequently interpreted as reflecting their expected activity in the organism. It may be questioned whether it is scientifically appropriate to use an organic solvent to extract hydrocarbons from particulate matter when the *in vivo* activity of the inhaled particulate matter is to be assessed for the living organism. Obviously, living matter does not have similar mechanisms which permit the separation of individual components analogous to solvent extraction *in vitro* and, therefore, their biological response *in vivo* will be primarily determined by the basic principles of bioavailability of chemical materials in living organisms.

Application of the microbial genetic assay to test dichloromethane extracts of hydrocarbons adsorbed on the surface of particles obtained from heavy duty diesel engines and unknown quality diesel fuels resulted in reports of positive mutagenic effects by several laboratories and premature suggestions that wider use of diesel engines on our roads may increase the risk of respiratory cancer in populations exposed to high concentrations of diesel emissions (*34*). However, the mutagenic effects of diesel particles vary with engine type and diesel fuels (*35*), as well as with the type of extraction solvent used (*36*), and both completely negative as well as highly positive values have been reported from different laboratories (*37*).

A minimum quality fuel with a low cetane value, high aromatic content and high nitrogen content produced the maximum mutagenic response in a comparative study. However, the measured concentration of 0.1 μg of benzo[a]pyrene per plate of this sample was not sufficient to explain the observed mutagenic effect (*35*). In addition, the response did not require activation with mammalian enzymes, another factor contradicting the major role of benzo[a]pyrene. Pitts (*38*) proposed that polycyclic aromatic hydrocarbons adsorbed on the surface of the particulate matter during the combustion process can react with other simultaneously emitted gaseous pollutants and form reaction products (nitroarenes) which are direct mutagens in the Ames test. Löfroth (*39*) and Rosenkranz (*40*) identified mutagenic nitropyrenes in xerographic toners containing carbon black. Pederson (*41*) studied the reactivity of diesel particulate extract with DNA and concluded that the behavior of the extract was more similar to nitroaromatic compounds than to unsubstituted benzo[a]pyrene. Increased mutagenicity under anerobiosis and decreased mutagenicity in bacteria lacking nitroreductase enzymes suggested that nitrocompounds are involved in the mutagenic activity of diesel particle extracts (*41*). Thin layer chromatography separation identified a major fraction of the activity in fractions associated with monosubstituted aromatic compounds. Absorption spectra indicated nitrosubstituted pyrene as the main nitroaromatic compound (*43*). Tests with recently developed dinitropyrene-resistant (Salmonella strains disclosed highly potent dinitrocompounds, 1,8-dinitropyrene and 1.6-dinitropyrene, as the predominant mutagenic components of the diesel particulate extract, in spite of their presence in concentrations lower than 1 ppm in diesel particulate (*44*).

Since nitroaromatic compounds seem to manifest their genotoxic properties only after the nitro groups have been activated, the presence of the nitroreductase enzyme is necessary for their mutagenic action. Fouts and Brodie (*45*) reported that the nitro-reductase enzyme system which converts nitro compounds into amines is present in mammalian tissues mainly in liver, partially in the kidney, but in traces or not at all in other tissues including the lung. The mutagenic activity of nitrocompounds observed in short-term microbial assay, therefore. may not be paralleled in the mammalian target tissues. Again, the potential for its manifestation would depend primarily on the fact that the active compounds can leave the particle and be distributed via systemic circulation to the liver.

In general, therefore, the scientific community did not disagree with the positivity of reported samples of diesel particulates in microbial tests but seriously questioned, as have many others, its significance in predicting long-term public health effects. First, positive mutagenic tests were observed only after adsorbed hydrocarbons had been stripped by powerful organic solvents and applied in the test in the form of extracts concentrated by evaporation.

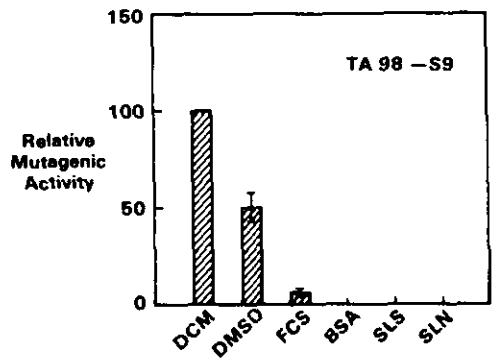

FIGURE 4. Comparison of the mutagenic activities of diesel particulate extracts by dichloromethane (DCM), dimethyl sulfoxide (DMSO), fetal calf serum (FCS), 0.5% bovine serum albumin, simulated lung surfactant (SLS) and saline (SLN). Data of Siak et al. (*46*).

Using the same laboratory method, Siak et al. (*46*) and Brooks et al. (*47*) demonstrated that when fluids have been used which are compatible with the internal environment of the human body instead of industrial organic solvents for extraction, mutagenic activity was significantly reduced and represented only a small fraction of the amount reported for the organic extracts. Figure 4 compares the mutagenic activity of a dichloromethane (solvent) extract with the activity of the same sample extracted by fetal calf serum, a solution of serum albumin, and a simulated lung surfactant. The mutagenic activity extracted from diesel particulates by typical body fluids such as blood serum or a solution of blood proteins is entirely negligible in comparison with that extracted by an organic solvent.

King et al. (*48*) confirmed that organic solvents are more efficient than physiological fluids in removing mutagens from diesel particles and reported also that the activity of hydrocarbons extracted with dichloromethane is greatly reduced upon addition of serum and lung cytosol. Subsequent incubation of serum and cytosol-bound organs with protease increased mutagenic activity, this prompted the authors to suggest that although serum or cytosol may partially remove mutagens from the particles, they remain firmly bound to proteins and do not exert biological activity of the degree observed after testing of dichloromethane extract.

Parallel studies conducted at other laboratories (*49*) also reported that organic materials dissociate from particles much more slowly *in vivo* than when extracted by organic solvents *in vitro* and that serum and tissue cytosols significantly reduce the cytotoxicity of diesel particle extracts (*50*). From 0 to about 8% of mutagenic activity extracted by dichloromethane was obtained by incubation of particles with biologically relevant solutions like lavage fluid or serum. Quantitative studies of the dissociation of benzo(a)pyrene from particles indicated that although 65% of the benzo[a]pyrene content was eluted by ethanol in 1 hr, none was eluted by saline, and only 12% was recovered after 24 hr perfusion of particles with 1:1 diluted serum. The authors concluded that biologically relevant solvents may bind or detoxify mutagenic compounds and make them unavailable for interaction with bacteria.

Biotransformation

It is well known that aromatic hydrocarbons are metabolized in the living organism by microsomal mixed function oxygenase to arene oxides, enzymatically hydrated to dihydrodiols, and further converted to catechols or conjugated with glutathione. Binding of reactive intermediates to cellular DNA was repeatedly proposed as a critical step in the observed genotoxic effects of polycyclic hydrocarbons (*51*, *52*) and the specific enzyme, aryl hydrocarbon hydroxylase, either is expected to activate or detoxify the effects of carcinogenic polycyclic hydrocarbons. Aromatic hydroxylase is present in many mammalian tissues, but the levels of its activity considerably vary among organs, strains and animal species (*53*).

Liver has the highest aromatic hydroxylase activity, but measurable enzyme levels were reported also in the lung and many other tissues, including alveolar macrophages (*54-59*). Tomingas (*54*) reported that alveolar as well as peritoneal macrophages can metabolize benzo[a]pyrene adsorbed on the surface of carbon particles, hematite or furnace dust. Dehnen (*60*) and Bast (*61*) described that aromatic hydroxylase in guinea pig macrophages can be induced, similarly as the liver and lung hydroxylase, by previous administration of polycyclic hydrocarbons. Increased hydroxylase activity was found also in alveolar macrophages lavaged from the lungs of smokers (*56-58*) and recently, McLemore (*62*) reported that although hydroxylase activity is higher in smokers than in nonsmokers, no difference was observed between smokers with and without neoplastic process in the lung. The capacity of tissues to induce higher levels of the hydroxylase activity, although different among individuals (*63*, *64*) is not a direct prerequisite for the neoplastic process. Neither does the formation of specific water-soluble metabolites of polycyclic hydrocarbons predict carcinogenic or mutagenic effects (*65*, *66*), and the data suggest that it may be difficult to

correlate the different *in vitro* assays with the events *in vivo* (*67*).

Theoretically, lung cells may extract, detoxify or activate mutagenic compounds independently of extracellular fluid, and make the *in vitro* system less applicable to the situation *in vivo*. This problem was addressed by Siak and Strom (*68*), who studied mutagenic properties of inhaled diesel particles that were deposited in the lung. Pulmonary alveolar macrophages were obtained by bronchopulmonary lavage from exposed animals immediately after exposure and 1, 4 and 7 days thereafter, concentrated by filtration and extracted with dichloromethane. When mutagenicity of diesel particle extracts collected from the inhaled air was used as a reference (Fig. 5), a positive mutagenic effect was detectable only in the extracts of macrophages obtained immediately and one day after exposure (Fig. 6). Starting with the second day after exposure, there was no mutagenic activity in extracts from macrophages, and in full agreement with the biological activity, the TLC fluorescence-banding pattern of the extracts completely disappeared. *In vitro* incubation of alveolar macrophages with diesel particles confirmed that the presence of macrophages reduces the mutagenic activity by more than 60% (*69*). Alveolar macrophages, which

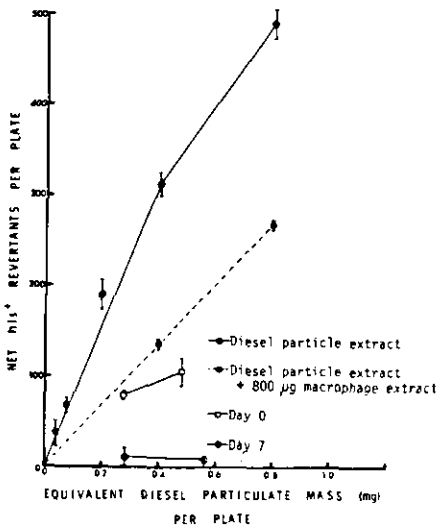

FIGURE 5. Mutagenic dose/response curves of diesel particle extracts obtained from diesel exhaust-exposed animals. Diesel particle extract and alveolar macrophage extracts were assayed for mutagenicity in tester strain TA98 without S9 enzyme activation: (—●—) diesel particle extract; (--●--) diesel particle extract plus 800 µg control macrophage extract; (—○—) macrophage extract from exposed rats immediately after exposure; (--○--) macrophage extract from exposed rats 7 days after exposure. Data of Siak and Strom (*68*).

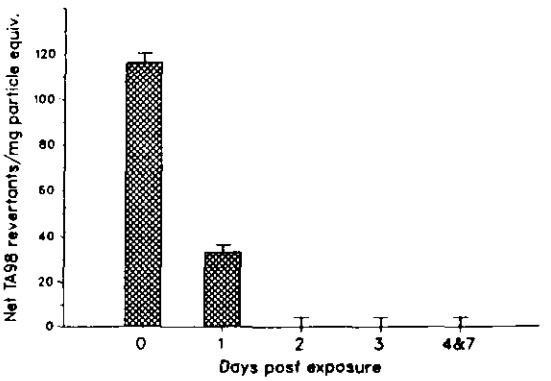

FIGURE 6. Post-exposure changes in mutagenic activity of inhaled diesel particles in alveolar macrophages of animals previously exposed to diluted diesel exhaust. Data of Siak and Strom (*68*).

accumulate most of the inhaled diesel particles from the respiratory tract, therefore, have a capacity to release or transform the fluorescent and mutagenic hydrocarbons within a relatively short period of time and, thus, significantly influence their biological activity in the respiratory system.

The fact that the alveolar macrophages can metabolize polycyclic aromatic hydrocarbons has been reported in the literature (*70*), and previous work in our laboratory demonstrated that mammalian liver enzymes activate the bacterial mutagenic activity of 1-nitropyrene and of diesel particle extract under specific laboratory condition (*71*). Therefore, an enzymatic transformation of the extractable organic compounds of diesel particles by macrophages may be one of the possible mechanisms involved. Another possible mechanism is the solubilization of the extractable organics from diesel particles by phospholipids from the lung surfactant and by other cellular components of the macrophage (*72-74*). The soluble complexes may diffuse into other tissues, and/or bind to other cellular constituents which render them unextractable by the method employed. Further *in vivo* and *in vitro* experiments are required to provide a better unoerstanding of the mechanisms involved, but the results thus far demonstrate that the insoluble particulates stored for a prolonged period of time in alveolar macrophages represent virtually an innocuous material which may have lost most of its biological activity (*75*).

The lack of biological activity of diesel particulates deposited in the respiratory tract was documented by the work of several laboratories. Chen et al. (*76*) investigated the effects of long-term inhalation of diluted diesel exhaust on aryl hydrocarbon hydroxylase activity and cytochrome P450

content in lung and liver microsomes in male Fischer-344 rats (*Rattus norvegicus*) and compared them with intraperitoneal and intratracheal administration of organic solvent extracts of hydrocarbon from the diesel particulates. Surprisingly, a decrease instead of an enzyme induction was observed in lung microsomal aromatic hydroxylase activity of animals after the full 9 months of exposure to diesel exhaust at the particulate concentration of 1500 $\mu g/m^3$ (Fig. 7). The observations were confirmed by other investigators (*77*). In contrast, 1.4- to 9-fold increases in aromatic hydroxylase activity were reported in liver and lung microsomes of rats pretreated by intraperitoneal doses of particulate extract, which were 10-15 times higher than the most conservative estimate of the deposited lung burden (25-125 mg/kg body weight). Similarly, direct intratracheal administration of the diesel particle extract (*78*) required doses as high as 6 mg/kg body weight before the activity of the induced enzyme in the lung was barely doubled (Fig. 8). The induction was slow and occurred selectively in the lung only, indicating that diesel particulate extract does not absorb easily into the lung circulation and is not distributed to other organs. The data suggest that the absence of enzyme induction in rat lung exposed to diesel exhaust is caused either by the inavailability of hydrocarbons for distribution in the body or by their presence in insufficient quantities for enzyme induction. The results indicate that inhaled diesel particles would not be capable of inducing aromatic hydroxylase in the lung unless the total deposited dose in the lung reaches approximately 6-8 mg of the particle extract per kilogram of body weight. Since the extractable portion represents only 10-15% of the total particulate mass, the required pulmonary deposits of diesel particles in a 70 kg man would be excessive to become a significant step in promotion of a potential neoplastic process.

Published data on a similarly negative immune response of the lymphoid tissues in the respiratory system to the presence of deposited particles are in good agreement with the observation of the lack of biological activity of the diesel particles during prolonged inhalation exposures (*79*). The inactivity of the sequestered particles is in sharp contrast with laboratory demonstrations that the diesel extract, when administered in excessive doses, produces positive effects in the immune response. Dziedzic (*79*) administered massive doses of dichloromethane extract of exhaust particles (10-50 mg/kg body weight, three times over 7 days, intraperitoneally) to mice (*Mus musculus*), and measured splenic lymphocyte response to the mitogens, lipopolysaccharide or concanavalin A. Mitogen reaction was determined in suspensions of lymphocytes from isolated spleens by culturing cells in the presence of a stimulating dose of lipopolysaccharide or concanavalin A. The cells were pulsed with tritiated thymidine, and the uptake of radioactivity was used as an index of response. The trend toward decreasing responsiveness in extract-injected animals is presented in Figure 9. In a separate experiment, T cell responsiveness of mice, similarly injected with extract to a contact hypersensitivity

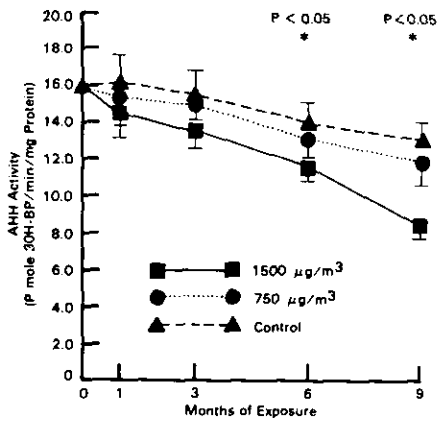

FIGURE 7. Pulmonary microsomal AHH activity ((ILLEGIBLE) mole 30 H-BP in g protein) of rats exposed to diluted diesel exhaust vs. months of exposure. Each symbol represents mean ± S.D. for six individual animals: (——) 1500 $\mu g/m^3$; (····) 750 μg^3; (----) control.

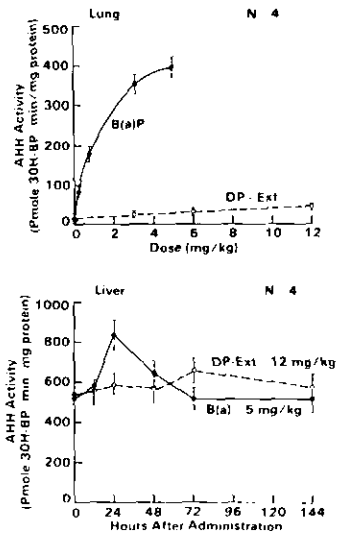

FIGURE 8. Lung and liver dose/response curves of microsomal aryl hydrocarbon hydroxylase (AHH) activity after intratracheal instillation of ben o(*a*)pyrene (B(a)P) and diesel particulate extract (DP-Ext). Symbols represent x¯p S.D. for four animals. Data of Chen and Vostal (*78*).

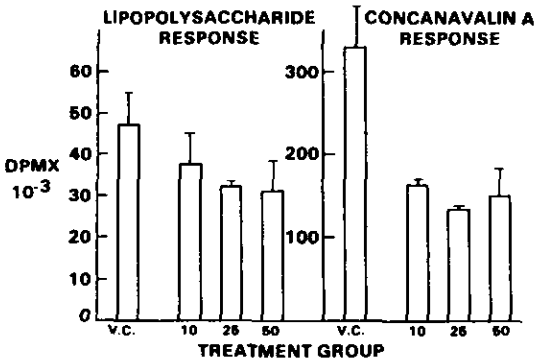

FIGURE 9. Splenic lymphocyte response to B-cell mitogen lipopolysaccharide or T-cell mitogen concanavalin A after *intraperitoneal injection of diesel particulate extract*. *VC* = vehicle control; 10, 25, 50 mg/kg dose. Mean ± S.E. Data of Dziedzic (*80*).

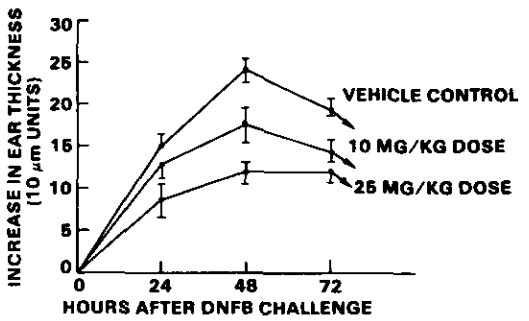

FIGURE 10. Ear thickness response to the sensitization challenge of dinitrofluorobenzene (0.5%) after intraperitoneally administered diesel particulate extract. Data of Dziedzic (*80*).

reaction was studied. In this experiment, groups of mice were sensitized with a 0.5% solution of dinitrofluorobenzene (DNFB) on a previously shaved abdomen. After 4 days, they were challenged on their left ears with the same solution; right ears were treated with vehicle alone. The increase in ear thickness at 24, 48 and 72 hr after challenge indicated a decreased ability to respond in the extract-treated animals (Fig. 10).

What appears to be evident from the data is that in contrast with the results of the laboratory tests *in vitro*, which may falsely lead to concerns about the potential neoplastic activity of the inhaled particles with polycyclic aromatic hydrocarbons, the real effect of particles is determined primarily by the availability of hydrocarbons for interaction with the sensitive cells of the respiratory tract. First, the living organism may not have identical mechanisms which will solubilize and elute the hydrocarbons from the surface of particles, similar to that of the powerful industrial solvents. Second, even if a prolonged residence time of particles could permit the solubilization of active mutagens from the particles, it remains to be seen if their mutagenic properties, as detected in a microbial system, are applicable to the completely different enzymatic conditions of the mammalian cell. Biological inactivity of the particulate deposits is well illustrated by the negative response of the inhaled particulates in the induction of metabolizing enzymes as well as by the completely negative immunological reaction and lack of significant functional or structural effects in long-term animal exposures to high concentrations of diesel particulates (*29*). In both cases, the biological response was clearly manifested when hydrocarbons were removed from the particles and administered in the form of particle-free extract; however, the effects were not observed after inhalation of particles with hydrocarbons adsorbed on the surface. Furthermore, the phagocytic function of the alveolar macrophage not only effectively prevents more intimate contact of inhaled particles with the sensitive cells of the respiratory system, but is capable of deactivating the biological aggressivity of the chemical materials adsorbed on their surface. Even if a long-term storage of the inhaled particles occurs in the respiratory system, it would primarily represent deposits of relatively innocuous material which might be more an indicator of the past exposure rather than an index of a clinically significant biological hazard.

In conclusion, studies conducted independently in several laboratories drew the same result: mutagenic components present on diesel particles are protein-bound or minimally soluble in biological fluids, and, therefore, not easily available for transfer into adjacent tissues or the systemic circulation. In this respect, the testing of the organic solvent extract *in vitro* does not represent the real biological activity of the diesel particles in the living organism. While the genotoxic effects observed after solvent extraction may represent significant scientific information, the data are not valid predictors of potential adverse effects of inhaled diesel particulates and cannot serve as a meaningful basis for the assessment of the hazards of diesel exhaust emissions in the human respiratory system. Unless the availability of the chemical compounds adsorbed on the surface of diesel particles to the biological fluids in the human body is considered in risk assessment, estimates of increased risk of lung cancer to diesel emissions will remain arbitrary and unrealistic.

REFERENCES

1. Ehrlich, P. Chemotherapeutics: scientific principles, methods and results. Lancet (2): 445 (1913).
2. Barr, W. H. Pharmacokinetic factors involved in the assessment of biological or physiologic availability. Drug Inform. Bull. (3): 27-45 (1969).
3. Gerarde, H. W., and Gerarde, D. S. The ubiquitous hydrocarbons. Assoc. Food Drug Officials U.S. Quart. Bull. 25: 161-172 (1961); ibid. 26:3-24 (1962); ibid. 26:65-77 (1962).
4. National Academy of Sciences. Vapor-Phase Organic Pollutants: Volatile Hydrocarbons and Oxidation Products. NRC-NAS Committee Report on Medical and Biologic Effects of Environmental Pollutants, National Academy of Sciences, Washington, DC, 1976.
5. National Academy of Sciences. Particulate Polycyclic Organic Matter. NRC-NAS Committee Report on Biologic Effects of Atmospheric Pollutants, National Academy of Sciences, Washington, DC, 1972.
6. U.S. DHEW. Smoking and Health, A Report of the Surgeon General. U.S. Dept. of Health, Education and Welfare, Public Health Service, Washington, DC, 1979, Publ. 79-50066.
7. Kuschner, M., Laskin, S., et al. Studies in pulmonary carcinogenesis. In: Inhalation Carcinogenesis (AEC Symposium Series No. 18), M. G. Hanna et al. (Eds.), U.S. AEC, Washington, DC, 1970, pp. 321-350.
8. Nettesheim, P., and Griesemer, R. A., Experimental models for studies of respiratory tract carcinogenesis. In: Pathogenesis and Therapy of Lung Cancer, Lung Biology in Health and Disease. C. Lenfant (Ed.), vol. 10, Dekker, New York, 1978, pp. 75-188.
9. Laskin, S., and Sellakumar, A. Models in chemical respiratory carcinogenesis. In: Experimental Lung Cancer: Carcinogenesis and Bioassays, E. Karbe and J. F. Park, (Eds.), Springer Verlag, New York, 1974, pp. 7-19.
10. Scala, R.A. Toxicology of PPOM. J. Occup. Med., 17: 784-788 (1975).
11. Buddingh, F., Bailey, M. J., Wells, B., and Haesemeyer, J. Physiological significance of benzo(α)pyrene adsorbed to carbon blacks: elution studies, AHH determinations. Am. Ind. Hyg. Assoc. J. 42: 503-509 (1981).
12. Huisingh, J. L., Bradow, R. L., Jungers, R. H., Harris, B. D., Zweidinger, R. B., Cushing, K. M., Gill, B. E., and Albert, R. E. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: study design, sample generation, collection and preparation. In: Health Effects of Diesel Engine Emissions, Vol. 2, W. E. Pepelko, R. M. Danner and A. N. Clarke (Eds.), U.S. EPA Cincinnati, OH, 1980, EPA 600/9-80-057a, pp. 788-799.
13. Williams, R. Unpublished data (1981).
14. National Academy of Sciences. Particulate Polycyclic Organic Matter. NAS/NRC, Washington, DC, 1972, p. 47.
15. Jackson, J. O., Warner, P. O., and Mooney, T. F. Profiles of benzo(a)pyrene and coal tar pitch volatiles at and in the immediate vicinity of a coke oven battery. Am. Ind. Hyg. Assoc. J. 35: 276-281 (1974).
16. Schulte, K. A., Larsen, D. J., Hornung, R. W., and Crable, J. V. Analytical methods used in a study of coke oven effluent. Am. Ind. Hyg. Assoc. J. 36: 131-139 (1975).
17. Grimmer, C., and Böhnke, H. The tumor-producing effect of automobile exhaust condensate and fractions thereof. Part I: Chemical studies. J. Env. Pathol. Toxicol. 1: 661-667 (1978).
18. Stenberg, U., Westerholm, P., Alsberg, T., Rannug, U., and Sundvall, A. Enrichment of PAH and PAH derivatives from automobile exhausts by means of a cryo-gradient sampling system. Paper presented at the Sixth International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, OH, October 27-29, 1981.
19. Griest, W. H., Jenkins, R. A., Pair, D. D., Quincy, R. B., Gill, B. E., and Reagan, R. R. Final Chemical Analyses of 2R1 Cigarette Smoke Condensate. U.S. EPA/DOE Fossil Fuels Research Materials Facility, Topical Report No. 25, April 1980.
20. Falk, H. L., Kotin, P., and Miller, A. Aromatic polycyclic hydrocarbons in polluted air as indicators of carcinogenic hazards. Int. J. Air Poll. (2): 202-209 (1960).
21. Falk, H. L., and Steiner, P. E. The adsorption of 3,4-benzpyrene and pyrene by carbon blacks. Cancer Res. 12: 40-43 (1952).
22. Falk, H. L., Miller, A., and Kotin, P. Elution of 3,4-benzpyrene and related hydrocarbons from soots by plasma proteins. Science 127: 474-475 (1958).
23. Kutsher, W., Tomingas, R., and Weisfeld, H. P. Untersuchungen über die Schädlichkeit von Russen unter Besonderer Berücksichtigung ihrer Cancerogenen Wirkung. 5. Uber die Ablösbarkeit von 3,4-Benzpyren durch Blutserum und einige Eiweissfaktoren des Serums. Arch. Hyg. Bakt. 151: 646-655 (1967).
24. Creasia, D. A., Poggenburg, J. K., Jr., and Nettesheim, P. Elution of benzo(a)pyrene from carbon particles in the respiratory tract of mice. J. Toxicol. Environ. Health 1: 967-975 (1976).
25. Medda, P. K., Dutta, S., and Dutta, S. Bioavailability of diesel particle bound ^{3}H-benzo(a)pyrene after intratracheal administration. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
26. Neal, J., Thornton, M., and Nau, C. A. Polycyclic hydrocarbon elution from carbon black or rubber products. Arch. Environ. Health, 4: 598-606 (1962).
27. Nau, C. A., Taylor, G. T., and Lawrence, C. H., Properties and physiological effects of thermal carbon black. J. Occup. Med. 18: 732-734 (1976).
28. Obrikat, H., and Wettig, K., Zur Löslichkeit polyzyklischer Kohlenwasserstoffe in Seren. Arch. Geschwulstforsch. 35: 326-337 (1970).
29. Vostal, J. J., Health aspects of diesel exhaust particulate emissions. Bull. N. Y. Acad. Med., 56: 914-934 (1980).
30. Williams, R., and Swarin, S. Benzo(a)pyrene Emissions from Gasoline and Diesel Automobiles. GM Research Laboratories Publication GMR-2881, Warren, MI, 1979.
31. Albert, R., U.S. EPA-CAG Initial Review on Potential Carcinogenic Impact of Diesel Engine Exhaust, Washington, DC, June 11, 1979.
32. Plaks, J., U.S. EPA Workshop on Carcinogen Risk Assessment of Diesel Engine Exhaust, Research Triangle Park, NC, February 24-25, 1981; Official Transcript, pp. 150-151, U.S. EPA, 1981.
33. Pederson, T., and Siak, J. Unpublished data.
34. Gage, S. J., Precautionary Notice on Laboratory Handling of Exhaust Products from Diesel Engines. Office of Research and Development, U.S. EPA, November 4, 1977.
35. Huisingh, J., Bradow, R., Jungers, R., Claxton, L., Zweidinger, R., Tejada, S., Bumgarner, J., Duffield, F., Waters, M., Simmon, V. F., Hare, C., Rodriguez, C., and Snow, L. Application of bioassay to the characterization of diesel particle emissions. In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures M. D. Waters, S. Nesnow, J. L. Huisingh, et al. (Eds.), Plenum Press, New York, 1978, pp. 381-418.

36. Taylor, G. T., Redington, T. E., Bailey, M. J., Buddingh, F., and Nau, C. A., Solvent extracts of carbon black determination of total extractables and analysis for benzo-(a)pyrene. Am. Ind. Hyg. Assoc. J. (41): 819-825 (1980).
37. McGrath, J., Schreck, R. M., and Siak, J.-S. Mutagenic Screening of Diesel Particulate Material. GM Research Publication No. 2743, Warren, MI, September 1978.
38. Pitts, J. N. Photo-chemical and biological implications of the atmospheric reactions of amines and benzo(a)pyrene. Phil. Trans. Roy. Soc. London, 290: 551-556 (1979).
39. Löfroth, G., Hefner, E., Alfheim, I., and Möller, M. Mutagenic activity in photocopiers. Science 209: 1037 (1980).
40. Rosenkranz, H. S., McCoy, E. C., Sanders, D. R., Butler, M., Kiriazides, D. K., and Mermelstein, R. Nitropyrenes, isolation, identification and reduction of mutagenic impurities in carbon black and toners. Science 209: 1039-1043 (1980).
41. Pederson, T. C. DNA-binding studies with diesel particulate extract. In: Health Effects of Diesel Engine Emissions, W. E. Pepelko, R. M. Danner and A. N. Clarke (Eds.), Cincinnati, OH, 1980, EPA Publ. 600/9-80-057a, pp. 481-497.
42. Pederson, T. C., and Siak, J.-S. Involvement of Nitro-Substituted PAH in the Mutagenic Activity Extracted from Diesel Exhaust Particles. Presented at the Society of Toxicology Meeting, San Diego, CA, 3 March (1980).
43. Pederson, T.C., and Siak, J.-S. Characterization of direct-acting mutagens in diesel exhaust particulates by thin-layer chromatography. Paper presented at the American Chemical Society Meeting, San Francisco, CA, August 24-29, 1980.
44. Pederson, T. C. Dinitropyrenes: their probable presence in diesel particle extracts and consequent effects on mutagen activations by NADPH-dependent S9 enzymes. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
45. Fouts, J. R., and Brodie, B. B. The enzymatic reduction of chloramphenicol, 1-nitrobenzoic acid and other aromatic nitrocompounds in mammals. J. Pharmacol. 119: 197-207 (1957).
46. Siak, J.-S., Chan, T. L., and Lee, P. S. Diesel particulate extracts in bacterial test systems. In: Health Effects of Diesel Engine Emissions, vol. 1, W. E. Pepelko, R. M. Danner and A. N. Clarke (Eds.), EPA, Cincinnati, OH, 1980, EPA-600/9-80-057a, pp. 245-262.
47. Brooks, A. L., Wolff, R. K., Royer, R. E., Clark, R., Sanchez, A., and McClellan, R. O. Biological availability of mutagenic chemicals associated with diesel exhaust particles. In: Health Effects of Diesel Engine Emissions, vol. 1, W. E. Pepelko, R. M. Danner and A. N. Clarke (Eds.), EPA, Cincinnati, OH, 1980, EPA Publ. 600/9-80-057a, pp. 345-358.
48. King, L. C., Kohan, M. J., Austin, A. C., Claxton, L. D., and Huisingh, J., Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. Environ. Mutagenesis 3: 109-121 (1981).
49. Li, A. P., Antagonistic effects of animal sera, lung and liver cytosols and sulfhyrdryl compounds on the cytotoxicity of diesel exhaust particle extracts. Toxicol. Appl. Pharmacol. 57: 55-62 (1981).
50. Li, A. P., Royer, R. E., Brooks, A. L., and McClellan, R. O. Cytotoxicity, mutagenicity and co-mutagenicity of diesel exhaust particle extracts on Chinese hamster ovary cells *in vitro*. Paper presented at EPA 1981 Diesel Emissions Symposium, Research Triangle Park, NC, October 5-7, 1981.
51. Sims, P., and Grover, P. L. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Adv. Cancer Res. 20: 165-274 (1974).
52. Gelboin, H. V., Selkirk, J., Okuda, T., Nemoto, N., Yang, S. K., Wiebel, F. J., Whitlock, J. P., Jr., Rapp, H. J., and Bast, R. C., Jr. Benzo(a)pyrene metabolism: enzymatic and liquid chromatographic analysis and application to human liver, lymphocytes and monocytes. In: Biological Reactive Intermediates, D. J. Jollow, J. J. Kocsis, R. Snyder, and H. Vainio (Eds.), Plenum Press, New York, 1977, pp. 98-123.
53. Nebert, D. W., and Gelboin, H. V. The *in vivo* and *in vitro* induction of aryl hydrocarbon hydroxylase in mammalian cells of different species, tissues, strains and developmental and hormonal status. Arch. Biochem. Biophys. 134: 76-89 (1969).
54. Tomingas, R., Dehnen, W., Lange, H. U., Beck, E. G., and Manojlovic, N. The metabolism of free and soot-bound benzo(a)pyrene by macrophages from guinea pigs *in vitro*. Zbl. Bakt. Hyg. I Abt. B155: 159-167 (1971).
55. Tomingas, R., Lange, H. U., Beck, E. G., Manojlovic, N., and Dehnen, W. The elution of benzo(a)pyrene adsorbed on particulates by macrophages cultured *in vitro*. Zbl. Bakt. Hyg. I Abt. B155: 148-158 (1971).
56. Cantrell, E., Busbee, D., Warr, G., and Martin, R. Induction of aryl hydrocarbon hydroxylase in human lymphocytes and pulmonary alveolar macrophages—a comparison. Life Sci. 13: 1649-1654 (1973).
57. Cantrell, E. T., Warr, G. A., Busbee, D. L., and Martin, R. R. Induction of aryl hydrocarbon hydroxylases in human pulmonary alveolar macrophages by cigarette smoking. J. Clin. Invest. 52: 1881-1884 (1973).
58. Harris, J. O., Swenson, E. W., and Johnson, J. E., III. Human alveolar macrophages: comparison of phagocytic ability, glucose utilization, and ultrastructure in smokers and nonsmokers. J. Clin. Invest. 49: 2086-2096 (1970).
59. Dehnen, W. Studies on the metabolism of benzo(a)pyrene in alveolar macrophages. I. Uptake of benzo(a)pyrene and induction of benzo(a)pyrene hydroxylase. Zbl. Bakt. Hyg. I. Abt. B160; 191-211 (1975).
60. Dehnen, W. Studies on the metabolism of benzo(a)pyrene in alveolar macrophages. II. Kinetics of metabolism and characterization of the metabolites. Zbl. Bakt. Hyg. I. Abt. B161: 1-25 (1975).
61. Bast, R. C., Jr., Shears, B. W., Rapp, H. J., and Gelboin, H. V., Aryl hydrocarbon (benzo(a)pyrene) hydroxylase in guinea pig peritoneal macrophages: benzo(a)anthracene-induced increase of enzyme activity *in vivo* and in cell culture. J. Natl. Cancer Inst. 51: 675-678 (1973).
62. McLemore, T. L., Martin, R. R., Busbee, D. L., Richie, R. C., Springer, R. R., Toppell, K. L., and Cantrell, E. T. Aryl hydrocarbon hydroxylase activity in pulmonary macrophages and lymphocytes from lung cancer and noncancer patients. Cancer Res. 37: 1175-1181 (1977).
63. Kellermann, G., Cantrell, E., and Shaw, C.R. Variations in extent of aryl hydrocarbon hydroxylase induction in cultured human lymphocytes. Cancer Res., 33: 1654-1656 (1973).
64. Rüdiger, H. W., Kohl, R., Mangels, W., von Wichert, P., Bartram, C. R., Wohler, W., and Passarge, E. Benzpyrene induces sister chromatid exchanges in cultured human lymphocytes. Nature 262: 290-292 (1976).
65. Kouri, R. E., McKinney, C., Sosnowski, R., and Schechtman, L. M., Factors influencing aryl hydrocarbon hydroxylase (AHH) activity in human lymphocytes. Proc. AACR 18: 150 (1977).
66. Jett, J. R., Moses, H. L., Branum, F. L., Taylor, W. F., and Fontana, R. S. Benzo(a)pyrene metabolism and blast transformation in peripheral blood mononuclear cells from smoking and nonsmoking populations and lung cancer patients. Cancer, 41: 192-200 (1978).
67. Bast, R. C., Bast, B. S., and Gelboin, H. V. Metabolism of

polycyclic aromatic hydrocarbons by lymphoreticular cells. In: Extrahepatic Metabolism of Drugs T. E. Gram, (Ed.), SP Med. Books, London, 1980.
68. Siak, J.-S., and Strom, K. A. Mutagenic activity of diesel particles in alveolar macrophages from rats exposed to diesel engine exhaust. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
69. King, L. C., Tejada, S. B., and Lewtas, J. Evaluation of the release of mutagens and 1-nitropyrene from diesel particles in the presence of lung macrophage cells in culture. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
70. Gram, T. E. The metabolism of xenobiotics by mammalian lung. In: Extrahepatic Metabolism of Drugs, T.E. Gram (Ed.), SP Med. Books, London 1980.
71. Pederson, T. C., and Siak, J.-S. The activation of mutagens in diesel particle extract with rat liver S9 enzymes. J. Appl. Toxicol. 1: 61-66 (1981).
72. Heath, D., Smith, P., and Hasleton, P. S. Effects of chlorphentermine on the rat lung. Thorax 28: 551-558 (1973).
73. Smith, P., Heath, D., and Hasleton, P. S. Electron microscopy of chlorphentermine lung. Thorax 28: 559-566 (1973).
74. White, H. J., and Garg, B. D. The participation of the pulmonary type II cell response to inhalation of diesel exhaust emission: late sequelae. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
75. Falk, H. L., Kotin, P., and Markul, T. The disappearance of carcinogens from soot in human lungs. Cancer 11: 482-488 (1958).
76. Chen, K.-C., and Vostal, J. J. Aryl hydrocarbon hydroxylase activity induced by injected diesel particulate extract vs. inhalation of diluted diesel exhaust. J. Appl. Toxicol. 1: 127-131 (1981).
77. Navarro, C., Charboneau, J., and McCauley, R. The effect of *in vivo* exposure to diesel exhaust on rat hepatic and pulmonary microsomal activities. J. Appl. Toxicol. 1: 124-126 (1981).
78. Chen, K.-C., and Vostal, J. J. The potential for aromatic hydroxylase induction in the lung by inhaled diesel particles. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.
79. Dziedzic, D. Differential counts of B and T lymphocytes in the lymph nodes, circulating blood and spleen after inhalation of high concentrations of diesel exhaust. J. Appl. Toxicol. 1: 111-115 (1981).
80. Dziedzic, D. The effects of diesel exhaust on cells of the immune system. Paper presented at EPA 1981 Diesel Emissions Symposium, Raleigh, NC, October 5-7, 1981.