

These data indicate that in this instance 1-NP and the DNPs did not contribute significantly to the induction of skin tumors in mice.

Of the compounds detected, BaP was the major contributor to the tumorigenic potency in the Nissan extract (Table C.3). Benzo[b]fluoranthene (BbF) and benzo[j]fluoranthene (BjF) were also significant contributors. In the case of the Volkswagen extract, the relatively low concentration of BaP relative to other PAHs skewed the contributions of BbF, BjF, and fluoranthene so that they were at least as significant as BaP in terms of tumorigenicity. It is important to note that DBAs and DBPs were not analyzed in these extracts.

Savard and coworkers (1992) have performed a detailed chemical analysis of a National Institute of Standards and Technology (NIST) standard reference material particulate matter (SRM 1650) from a heavy-duty diesel engine (May et al. 1992). Estimates of the relative tumorigenic contributions of PAHs and nitro-PAHs are presented in Table C.4. Benzo[j]fluoranthene made a significant contribution to the preweanling mouse assay, but had only a slight effect in the lung implantation assay.

ABBREVIATIONS

BaA	benz[a]anthracene
BaP	benzo[a]pyrene
BbF	benzo[b]fluoranthene
BjF	benzo[j]fluoranthene

BkF	benzo[k]fluoranthene
CPP	cyclopenta[c,d]pyrene
DBA	dibenzanthracene
DBaC	dibenz[a,c]anthracene
DBaH	dibenz[a,h]anthracene
DBaJ	dibenz[a,j]anthracene
DBP	dibenzopyrene
DBaH	dibenzo[a,h]pyrene
DBaI	dibenzo[a,i]pyrene
DEN	diethylnitrosamine
DNP	dinitropyrene
1,6-DNP	1,6-dinitropyrene
1,8-DNP	1,8-dinitropyrene
DPN	dipentyl nitrosamine
Fe <sub>2</sub> O <sub>3</sub>	ferric oxide
IcdP	indeno[1,2,3-cd]pyrene
6-NC	6-nitrochrysene
NIST	National Institute of Standards and Technology
NTP	National Toxicology Program
PAC	polynuclear aromatic compound
PAH	polycyclic aromatic hydrocarbon
SRM	Standard Reference Material
TiO <sub>2</sub>	titanium dioxide
TPA	tetradecanoyl phorbol acetate
1-NP	1-nitropyrene

Table C.4. Relative Contributions to Tumorigenicity of Polycyclic Aromatic Hydrocarbons and Nitro-Polycyclic Aromatic Hydrocarbons in Heavy-Duty Diesel Engine Emissions<sup>a</sup>

Compound (µg/g)	Concentration Relative to Benzo[a]pyrene	Comparative Potency Relative to Benzo[a]pyrene		
		Prewanling Mouse (BLU:Ha) Assay	Lung Implantation Assay	Skin-Painting Assay
Fluoranthene (51)	42.50	0.85	—	—
BaA (6.5)	5.42	0.76	—	0.05
Chrysene (22)	18.33	0	—	1.28
BbF (12.0) <sup>b</sup>	10.00	2.10	1.10	3.50
BjF (10.0) <sup>b</sup>	8.33	3.67	0.25	0.67
BkF (2.1)	1.75	0.05	0.05	0.04
<b>BaP (1.2)</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>	<b>1.00</b>
IcdP (2.1)	1.75	0	0.14	0.02
DBaI (3.3) <sup>b</sup>	2.75	1.00	—	5.78
DBaH (0.7)	0.58	8.40	—	1.11
1-NP (19)	15.83	0	0	0
Total -BaP		16.83	1.54	12.45

<sup>a</sup> Particle extract from SRM 1650 (5.7-L Caterpillar engine) (May et al. 1992).

<sup>b</sup> Data are from Savard et al. (1992).

# Genotoxicity of Diesel Emissions

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## Part I: Mutagenicity and Other Genetic Effects

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Introduction	223
Genotoxicity of Diesel Emissions in Various Bioassays	223
Bacterial Mutation Assays	223
Mammalian Mutation Assays	226
Sister Chromatid Exchange	227
Micronucleus Test	227
Chromosome Aberrations	227
Heritable Mutations	227
Other DNA or Chromosome Damage Assays	227
Cell Transformation Assays	228
DNA Adducts	228
Mutagenic Compounds in Diesel Emissions	232
Particle-Associated Organic Compounds	232
Vapor-Phase Compounds	235
Summary	236
References	236
Abbreviations	242



## INTRODUCTION

Diesel exhaust is a complex mixture of gases, vapors, and particulate matter that results from the incomplete combustion of diesel fuel and lubricants (International Agency for Research on Cancer 1989). In regard to health effects, the key component of concern for inhaled diesel exhaust is particulate matter, which diesel engines emit in much greater quantities than contemporary gasoline engines of comparable size (International Agency for Research on Cancer 1989). Diesel exhaust particulate matter, which consists of chain aggregates and clusters of carbonaceous particles with a high surface area, includes mutagenic and carcinogenic organic compounds, inorganic salts, and metals adsorbed on its surface. When the exhaust stream leaves the high-temperature environment of the exhaust pipe and is diluted with cooler air, polycyclic aromatic hydrocarbons (PAHs)\* with four or five rings condense onto the surface of exhaust particles. Particles in diluted diesel exhaust are of a respirable size, and particles inhaled by humans are expected to deposit in the lung airways (Xu and Yu 1987; Scheepers and Bos 1992a,b). These particles carrying the condensed polycyclic aromatic compounds have been the subject of intense investigation during the last two decades because it has been suggested, on the basis of their genotoxic properties, that the organic compounds play a role in tumor induction.

Assays for genotoxicity provide not only information regarding suspected carcinogens but also insights into the mechanisms of carcinogenicity, reproductive toxicity, and other genetically influenced processes caused by chemicals that react with the DNA. According to the electrophilic chemical carcinogenesis theory, reactive chemicals and their "activated" metabolites form products with DNA called adducts, and these products, if not repaired by an error-free mechanism, can cause mutations or chromosomal alterations during cell replication. Such effects are thought to be responsible in part for the carcinogenicity of DNA-reactive agents (Weinberg 1991). The organic chemicals bound to the carbon core of diesel exhaust particles have been hypothesized to induce mutations in critical genes, thus initiating the process of carcinogenesis. Because a large number of rodent carcinogens are mutagenic in *in vitro* bioassays (Piegorsh and Hoel 1988; Rosenkranz and Ennever 1990), efforts to understand the mechanism of tumor development have concentrated on isolating and characterizing the principal particle-associated mutagens.

The amount and types of chemicals present in diesel exhaust are a function of many factors, including engine

design and maintenance, engine fuel and lubricating oil composition, and engine operating conditions (International Agency for Research on Cancer 1989). Mutagenicity assays have been used to determine the influence of these factors on the formation of mutagens in diesel exhaust (reviewed by International Agency for Research on Cancer 1989) and the extent of reduction in mutagenic emissions as a result of control technology (discussed in the background paper by Sawyer and Johnson, this report).

This background paper describes the genotoxicity of diesel exhaust in *in vitro* and *in vivo* bioassays and characterizes the mutagenic chemicals present in the exhaust.

## GENOTOXICITY OF DIESEL EMISSIONS IN VARIOUS BIOASSAYS

A detailed review of data on the genotoxicity of diesel exhaust from various bioassays conducted prior to 1988 was prepared by the International Agency for Research on Cancer (1989). Extracts of diesel exhaust particles in organic solvents, gas-phase components, or particles themselves have been shown to be genotoxic in a variety of short-term bioassays as summarized in Table 1. Because the *Salmonella typhimurium* mutation assay has been used most widely to study the mutagenicity of diesel exhaust and, when combined with analytical methods, to identify key mutagenic compounds, the general principle of this assay is discussed briefly. A detailed description of this assay and the other assays discussed below is available elsewhere (International Agency for Research on Cancer 1980).

## BACTERIAL MUTATION ASSAYS

The first mutagenicity tests of diesel exhaust particle extracts were carried out by Huisingsh and associates (1978) using the *S. typhimurium* mutagenicity assay (McCann et al. 1975). This assay measures the ability of substances to induce mutations in the bacterium *S. typhimurium* as a result of DNA alterations associated with substances in the emissions. Thus a positive response in the *Salmonella* mutagenicity assay indicates that the material tested is mutagenic and has the potential for causing cancers.

Many chemicals are mutagenic in *S. typhimurium* only when a preparation of rat liver postmitochondrial homogenate called S9 is added to the bacterial incubation mixture. This is because the S9 fraction contains enzymes that metabolize the chemicals into reactive substances (electrophiles) that have the capacity to modify DNA. Compounds that require S9 for manifestation of their mutagenic activity are termed indirect-acting mutagens and include most PAHs

\* A list of abbreviations appears at the end of this paper.

**Table 1.** Summary of Results of Selected Short-Term in Vitro and in Vivo Bioassays Used to Evaluate the Genotoxicity of Diesel Exhaust and Diesel Exhaust Constituents<sup>a</sup>

Assay	Whole Diesel Exhaust	Particulate Matter	Particulate Matter Extracts	Filtered Exhaust	References
<b>Bacterial Mutation</b>					
<i>Salmonella typhimurium</i> (His, Trp) <sup>b</sup>				+	Stump et al. 1982; Green et al. 1983; Rannug et al. 1983; Matsushita et al. 1986; Bagley et al. 1987, 1993; Ong et al. 1985
			+		Huisingh et al. 1978; Clark and Vigil 1980; Lewtas 1983; Keane et al. 1991; Crebelli et al. 1991; Mauderly et al. 1994
		+			Brooks et al. 1980; King et al. 1981; Siak et al. 1981; Belisario et al. 1984; Wallace et al. 1987; Willems et al. 1989; Keane et al. 1991; Gu et al. 1992; Schenker et al. 1992
<i>Escherichia coli</i> WP2; K12 (Trp) <sup>b</sup>			+		Lewtas 1983; Lewtas and Williams 1986; Crebelli et al. 1991
<b>Mammalian Mutation</b>					
Mouse BALB/c3T3 fibroblasts (ATPase) <sup>b</sup>			±		Curren et al. 1981; Lewtas 1983
Mouse lymphoma L5178Y cells (TK) <sup>b</sup>			+		Rudd 1980; Mitchell et al. 1981; Lewtas 1983
Chinese hamster ovary cells (HPRT) <sup>b</sup>			±		Brooks et al. 1980; Casto et al. 1980; Li and Royer 1982
Chinese hamster ovary cells (HPRT) <sup>b</sup>		+			Chescheir et al. 1981
Chinese hamster V79 cells (HPRT) <sup>b</sup>			±		Rudd 1980; Morimoto et al. 1986
Human lymphoblasts (TK) <sup>b</sup>			+		Liber et al. 1981; Barfknecht et al. 1982
<b>Chromosome Aberrations in Vitro</b>					
Chinese hamster V79 cells			±		Casto et al. 1981; Hasegawa et al. 1988
Chinese hamster ovary cells			+		Lewtas and Williams 1986
Human lymphoblasts			+		Lewtas and Williams 1986
<b>Chromosome Aberrations in Vivo</b>					
Mice bone marrow	-				Pepelko and Peirano 1983
Human lymphocytes	-				Nordenson et al. 1981; Fredga et al. 1982
<b>Heritable Mutations</b>					
<i>Drosophila</i> , sex-linked recessive	-				Schuler and Niemeier 1981
Mice, dominant lethal test	-				Pepelko and Peirano 1983

(Table continues next page.)

**Table 1.** Summary of Results of Selected Short-Term in Vitro and in Vivo Bioassays Used to Evaluate the Genotoxicity of Diesel Exhaust and Diesel Exhaust Constituents<sup>a</sup> (continued)

Assay	Whole Diesel Exhaust	Particulate Matter	Particulate Matter Extracts	Filtered Exhaust	References
<b>Other DNA and Chromosome Damage Tests</b>					
<i>Saccharomyces cerevisiae</i> , mitotic recombination			+		Lewtas 1983
Unscheduled DNA synthesis, rat tracheal cells		+			Kawabata et al. 1986
<b>Cell Transformation Assays</b>					
Mouse BALB/c3T3 cells			+		Curren et al. 1981; Hasegawa et al. 1988
Syrian hamster embryo cells + SA7 virus			+		Casto et al. 1981
<b>Sister Chromatid Exchanges in Vitro</b>					
Chinese hamster ovary cells			+		Hasegawa et al. 1988
Human peripheral cells	±		+		Tucker et al. 1986
Human lymphocytes			+		Morimoto et al. 1986
Chinese hamster V79 cells		+	+		Keane et al. 1991
<b>Sister Chromatid Exchanges in Vivo</b>					
B6C3F <sub>1</sub> mice, bone marrow	-		+	+	Pereira 1982
Rat bone marrow or white blood cells	-				Morimoto et al. 1986; Mauderly et al. 1994
Syrian hamster lung cells		+			Guerrero et al. 1981
Syrian hamster fetal liver cells	-	-	+		Pereira 1982
<b>Micronucleus Assay in Bone Marrow</b>					
Mice, rats	-				Morimoto et al. 1986; Lewis et al. 1986; Ong et al. 1985; Mauderly et al. 1994
Mice, Chinese hamster erythrocytes	±	+	-		Pereira 1982
Chinese hamster V79 cells and ovary cells	-	+	+		Gu et al. 1992

<sup>a</sup> Table does not include results of bioassays conducted on extracts that were fractionated. + = positive result; - = negative results; ± = mixed results; blank = no data available.

<sup>b</sup> Mutations are assessed at the gene locus indicated within parentheses: His = histidine independence as mutation indicator; Trp = tryptophan independence as mutation indicator; TK = thymidine kinase; HPRT = hypoxanthine phosphoribosyl transferase; ATPase = adenosine triphosphatase.

(International Agency for Research on Cancer 1983). In contrast, some chemicals do not require external metabolic activation by S9 to manifest their mutagenicity; these are termed direct-acting mutagens. Examples of this class of compounds found in diesel exhaust extracts are the nitro-PAHs. These chemicals are metabolized to mutagenic substances by enzymes called nitroreductases that are present in the bacteria (Mermelstein et al. 1981).

The *Salmonella* mutagenicity assay is conducted using different strains of bacteria (e.g., TA 98, TA 100), allowing

different types of mutations to be distinguished from one another. This information, together with the differential response (e.g., with or without metabolic activation), provides some understanding of the general classes of chemicals causing the mutagenic response. Other *Salmonella* tester strains such as TA 98NR and TA 98DNP<sub>6</sub> (Rosenkranz et al. 1982; McCoy et al. 1983) have been developed to differentiate among the responses of nitro-PAHs. TA 98NR and TA 98DNP<sub>6</sub> are deficient in nitroreductases and *o*-transacetylases, respectively. These enzymes are required

to convert nitro-PAHs into DNA-reactive metabolites capable of causing mutations. Characteristically, the nitro-PAHs yield reduced mutagenic responses in TA 98NR and TA 98DNP<sub>6</sub> when compared with the parent strain TA 98, which contains all the enzymes required for activation.

As indicated in Table 1, most bacterial mutation assays of diesel exhaust constituents have been conducted with organic solvent extracts of diesel exhaust particles. The solvent-extractable organic material is generally direct-acting, which indicates that the mutagenicity is primarily due to nitro-PAHs (Rosenkranz et al. 1983). Diesel exhaust extracts have also been demonstrated to be mutagenic in other bacteria, such as *Escherichia coli* WP2 (Lewtas and Williams 1986), WP2uvra (Crebelli et al. 1991), and *E. coli* K12 (Lewtas 1983). Except for *E. coli* K12, mutagenic activity was observed without exogenous metabolic activation. The vapor phase of diesel exhaust showed a mutagenic response in the bacterial assay in the presence of exogenous metabolic activation (Stump et al. 1982; Rannug et al. 1983; Matsushita et al. 1986; Bagley et al. 1987, 1993). One diesel exhaust particulate matter sample collected by electrostatic precipitation from a diesel automobile and added directly to the assay system without prior extraction in organic solvents exhibited mutagenicity in *Salmonella* strains in the presence and absence of exogenous metabolic activation (Belisario et al. 1984).

Many investigators have used bacterial bioassays to evaluate the bioavailability of mutagenic material under normal physiological conditions. Early studies indicated that eluting mutagens from diesel exhaust is difficult. When incubated with saline, serum, lung surfactant, or lung lavage fluids, diesel exhaust particles released little or no mutagenic activity (Brooks et al. 1980; King et al. 1981; Siak et al. 1981). Wallace and colleagues (1987), however, found that mutagens could be eluted from diesel exhaust with dipalmitoyl lecithin, a lung surfactant. Keane and coworkers (1991) reported that diesel exhaust particles dispersed in an aqueous mixture containing dipalmitoyl phosphatidyl choline, a major component of pulmonary surfactant; a sample extracted from the same source with dichloromethane exhibited similar mutagenic responses in the *Salmonella* mutagenicity and the sister chromatid exchange (SCE) assays. After the samples were separated into supernatant and sediment fractions, the activity of both the diesel exhaust samples was shown to reside exclusively in the sedimented fraction for the surfactant-dispersed sample and in the supernatant fraction for the solvent-extracted sample. The investigators concluded that genotoxic activity associated with diesel exhaust particles inhaled into the lungs may be

made bioavailable by virtue of the solubilization and dispersion properties of pulmonary surfactant components. A similar conclusion was made by Gu and coworkers (1992). However, the relevance of the bioavailability of genotoxic compounds in these in vitro systems to that in human lungs remains to be investigated.

Other investigators have studied the mutagenicity of urine samples of animals or humans exposed to diesel exhaust. The urine of Swiss Webster mice exposed to whole diesel exhaust (6 to 7 mg/m<sup>3</sup>, five days/week for seven weeks; Pereira 1982) or F344 rats exposed to diesel exhaust particles (1.9 mg/m<sup>3</sup> for 3 to 24 months; Ong et al. 1985) was not mutagenic to *S. typhimurium*. Belisario and coworkers (1984, 1985), however, found that urine from Sprague-Dawley rats administered diesel exhaust particles by gastric intubation or by intraperitoneal or subcutaneous administration was mutagenic in the *Salmonella* mutagenicity assay. Willems and associates (1989) studied office workers exposed to diesel exhaust and a group of car mechanics presumed to be exposed to high concentrations of diesel exhaust in their workplace and found no enhanced incidence or degree of either fecal or urinary mutagenicity, nor did the research reveal any relation between mutagenicity and a number of dietary variables that were studied. In this study, however, the investigators characterized diesel exhaust exposure based only on the observation that the mutagenic response to extracts of airborne particles collected from the garage area was stronger than the response to particle extracts collected outside the garage. No attempt was made to measure the actual exposure concentrations. After making adjustments for smoking, Schenker and colleagues (1992) found no significant evidence of an association between exposure to diesel exhaust and postshift urinary mutagenicity in a group of railroad workers.

#### MAMMALIAN MUTATION ASSAYS

As shown in Table 1, organic extracts of diesel exhaust particles are mutagenic in several mammalian systems (Chinese hamster ovary [CHO] cells, V79 Chinese hamster lung cells, mouse BALB/C 3T3 cells, and human lymphoblasts). Mutagenic effects in human lymphoblasts were observed only in the presence of exogenous metabolic activation (Liber et al. 1981; Barfknecht et al. 1982). Positive responses in other mammalian cell lines were also observed either in the presence or in the absence of metabolic activation. One study (Chescheir et al. 1981) showed that diesel exhaust particles caused mutations in CHO cells at the hypoxanthine phosphoribosyl transferase (HPRT) locus and that extraction with organic solvents was not necessary.

### SISTER CHROMATID EXCHANGE

Several in vitro studies conducted with extracts from diesel exhaust particles in cultured CHO cells and human lymphocytes have shown an increase in SCE (Table 1). Extracts of particulate matter from light-duty engines induced much higher SCE levels than extracts from heavy-duty engines in both CHO cells (Hasegawa et al. 1988) and cultured human lymphocytes (Morimoto et al. 1986). As noted above, Keane and coworkers (1991) reported that diesel exhaust particles disperse in aqueous mixtures of dipalmitoyl phosphatidyl choline and found that a sample extracted from the same source with dichloromethane and transferred into dimethylsulfoxide produced SCE in V79 cells. In a study by Tucker and associates (1986), cultured human lymphocytes from four healthy nonsmokers were exposed to diesel exhaust and SCE frequencies were measured. Cells from two of the four samples showed a statistically significant increase in SCE frequency.

A number of in vivo tests for SCE have been conducted following inhalation of diesel exhaust by test animals. No significant increase in the frequency of SCE was observed after exposure of BC6F<sub>1</sub> mouse bone marrow cells to diesel exhaust particles (12 mg/m<sup>3</sup> for 1 month) (Pereira 1982), of rats to diesel exhaust particles (4.0 mg/m<sup>3</sup> for 30 months) (Morimoto et al. 1986), or of F344/N rat white blood cells to diesel exhaust particles (2.5 and 6.5 mg/m<sup>3</sup> for 3 months) (Mauderly et al. 1994). Significant increases in SCE levels were observed, however, in the lung cells of Syrian hamsters exposed to diesel exhaust particle concentrations of 12 mg/m<sup>3</sup> for 3.5 and 8.5 months, but not at concentrations of 6 to 7 mg/m<sup>3</sup> for 3 months (Pereira 1982). Similarly, SCE frequency increased significantly in mouse bone marrow and lung cells after intratracheal administration of diesel exhaust particles or particulate extracts (Pereira 1982). No changes in SCE levels were observed in the fetal liver of pregnant Syrian hamsters exposed to either diesel exhaust particles (12 mg/m<sup>3</sup>) or to diesel particulate extracts at a lethal dose for 50% of the population (i.e., LD<sub>50</sub>) of 300 mg/kg of body weight (Pereira 1982). Administration of diesel extract, however, resulted in a dose-dependent increase in SCE levels in fetal liver (Pereira 1982). Guerrero and associates (1981) observed a dose-dependent increase in SCE levels after they instilled diesel exhaust particles intratracheally in Syrian hamsters.

### MICRONUCLEUS TEST

As shown in Table 1, micronuclei formation was not induced when ICR mice inhaled diesel exhaust at doses of 0.4 and 2.0 mg/m<sup>3</sup> for 4 to 18 months (Morimoto et al. 1986)

or at 2 mg/m<sup>3</sup> for 6 months (Ong et al. 1985; Lewis et al. 1986). Negative responses were also reported in studies with mice and Chinese hamsters exposed by inhalation to diesel exhaust particles at 6 or 12 mg/m<sup>3</sup> for one month (Pereira 1982) and 2.5 and 6.5 mg/m<sup>3</sup> for three months (Mauderly et al. 1994). Pereira (1982), however, observed a significant increase in micronuclei at the 6.0 mg/m<sup>3</sup> dose after six months. Intraperitoneal injection of diesel exhaust particles or particulate matter extracts yielded a negative response in both mice and hamsters (Pereira 1982), whereas diesel exhaust particles or their extracts reportedly increased the levels of micronuclei in cultured V79 and CHO cells (Gu et al. 1992).

### CHROMOSOME ABERRATIONS

As shown in Table 1, organic solvent extracts of diesel exhaust particles from a light-duty engine induced chromosome aberrations in V79 Chinese hamster cells (Hasegawa et al. 1988), CHO cells, and human lymphoblasts (Lewtas and Williams 1986). Extracts of particles from a heavy-duty diesel engine (Hasegawa et al. 1988) and a light-duty diesel engine (Casto et al. 1981), however, did not induce chromosomal aberrations in V79 cells and Syrian hamster cells, respectively.

No increase in the frequency of chromosome aberrations was noted either in a group of Swedish miners (Nordenson et al. 1981) or in a group of diesel truck drivers (Fredga et al. 1982) presumably exposed to diesel exhaust (Table 1). In these studies, measurements of carbon monoxide and nitrogen dioxide were used as measurements of diesel exhaust.

### HERITABLE MUTATIONS

Exposure to diesel exhaust did not induce heritable mutations in *Drosophila* using a sex-linked recessive lethal assay (Schuler and Niemeier 1981). In addition, induction of dominant lethal mutations was not evident in mice exposed by inhalation to 2 mg/m<sup>3</sup> of diesel exhaust for six months (Pepelko and Peirano 1983) (see Table 1).

### OTHER DNA OR CHROMOSOME DAMAGE ASSAYS

Kawabata and coworkers (1986) found that suspensions of diesel exhaust particles added to cultured rat tracheal ring cells induced unscheduled DNA synthesis. In another study, mitotic recombinations were observed in yeast (*Saccharomyces cerevisiae*) cells treated with diesel exhaust extracts (Lewtas and Williams 1986; Table 1).

### CELL TRANSFORMATION ASSAYS

In a study conducted by Curren and associates (1981), particulate extracts from a light-duty diesel engine transformed mouse BALB/C 3T3 cells (i.e., there was a loss of contact inhibition resulting in an ability to grow in soft agar or altered morphology); the activity was observed only in the presence of exogenous metabolic activation. Casto and colleagues (1981) found that extracts of diesel exhaust particles from three of four diesel engines enhanced transformation of Syrian hamster embryo cells in the presence of simian adenovirus (SA7). Hasegawa and coworkers (1988) showed that particle extracts from light-duty diesel engine exhaust were more potent inducers of cell transformation in mouse BALB/C 3T3 cells than extracts of exhaust particles from a heavy-duty diesel engine (Table 1).

### DNA ADDUCTS

The reaction between genotoxic chemicals and DNA to form covalently bonded reaction products (i.e., DNA adducts) is thought to be a key step in the initiation of cancer (Hemminki 1993). Measuring DNA adducts in target tissues or in surrogate tissues has been used to detect and monitor human exposures to genotoxic chemicals (International Agency for Research on Cancer 1988; dell'Omo and Lauwerys 1993), including some studies of the nitro-PAHs present in diesel exhaust (Smith et al. 1993). Several studies of DNA adducts have been conducted in animals exposed to diesel exhaust either by inhalation or by skin painting with extracts of diesel exhaust particulate matter. These studies have been conducted to determine whether exposure to diesel exhaust or its constituents induces formation of DNA adducts in lung and other tissues, and whether any evidence suggests that adducts can be related to tumorigenesis. The results of these studies are summarized in Tables 2 and 3.

The  $^{32}\text{P}$ -postlabeling assay (Reddy and Randerath 1986) is the most widely used method for measuring DNA adducts. In this procedure, DNA is hydrolyzed to 3'-nucleotides by treatment with enzymes. Adducted nucleotides are selectively enriched over normal nucleotides either by butanol extraction or by treatment with nuclease P1; butanol extraction selects adducted nucleotides with the aid of a phase-transfer agent, *tert*-butyl ammonium chloride (Gupta et al. 1985); nuclease P1 allows enrichment of adducted nucleotides by preferentially dephosphorylating normal nucleotides. Adducted nucleotides are then  $^{32}\text{P}$ -postlabeled with [ $\gamma$ - $^{32}\text{P}$ ] ATP and polynucleotide kinase. The resulting bisphosphate derivatives are separated by polyethyleneimine-cellulose thin-layer chromatography (Reddy and Randerath 1986).

The following questions can be addressed with these DNA adduct studies, as highlighted in the discussion below:

- Is there an increase in total DNA adducts in treated versus untreated animals?
- How does the adduct level change with the time course of exposure to diesel exhaust?
- Are specific adducts that are absent in untreated animals induced by exposure to diesel exhaust?
- How does the route of administration affect the levels of adducts?
- Because rat cancer bioassays indicate that the particulate matter in diesel exhaust is responsible for the induction of lung tumors, does exposure to carbon black, which is devoid of soluble PAHs, also result in the formation of DNA adducts in experimental animals?
- What evidence is there to suggest that DNA adducts induced by diesel exhaust are actually involved in tumorigenesis?

### Total Adducts

As shown in Table 2, results of the inhalation experiments do not show unequivocally that exposure to diesel exhaust is associated with the induction of DNA adducts in exposed animals. Early studies (Wong et al. 1986; Bond et al. 1988, 1990a) report small but significant increases in total DNA adduct levels as a result of exposure to diesel exhaust. For instance, analysis of lung tissue isolated from rats exposed by inhalation to diesel exhaust particle concentrations of  $10\text{ mg/m}^3$  for 12 weeks yielded a relative adduct labeling (RAL) of 5.0 adducts/ $10^9$  nucleotides in control animals versus a RAL of 14.0 adducts/ $10^9$  nucleotides in treated animals (Bond et al. 1990a). In studies conducted with diesel exhaust and an insoluble particle (i.e., carbon black), the findings were mixed: Bond and coworkers (1990b) reported a significant increase in total DNA adduct levels with both types of particles; however, Wolff and coworkers (1990) reported a significant increase only with diesel exhaust particles. In two recent studies (Mauderly et al. 1994; Randerath et al. 1995) of rats exposed to a diesel exhaust or carbon black particle concentration of  $6.5\text{ mg/m}^3$ , only rats exposed to diesel exhaust showed a significant increase in total adduct levels over controls at three months.

It should be noted, however, that the increases in the adduct levels induced by diesel exhaust in the above experiments are very small compared with those induced by carcinogens present in diesel exhaust (Smith et al. 1993). Moreover, Gallagher and coworkers (1994) reported no changes in total levels of DNA adducts induced by diesel exhaust, carbon black, or titanium dioxide ( $\text{TiO}_2$ ) particles at multiple time points when compared with control animals.

No firm conclusions can be drawn from these studies. The differences in the results may be related in part to different time points and the different tissues or cells (e.g., whole lung, type II cells, peripheral lung cells) selected for DNA adduct analysis, or the contrasting results may be attributable to subtle variations in the chromatographic procedures involved in DNA adduct analysis (International Agency for Research on Cancer 1993).

### Time Course

Bond and coworkers (1990a) conducted time-course measurements of DNA adducts in peripheral lung cells of rats and reported that adduct formation was independent of the exhaust concentration and that steady-state levels of DNA adducts were reached after prolonged exposure. Randerath and colleagues (1995) observed that diesel exhaust exposure at a concentration of  $6.5 \text{ mg/m}^3$  increased the total

**Table 2.** Summary of DNA Adduct Studies: Inhalation of Diesel Exhaust and Other Particulate Matter

Reference	Substance (concentration)	Exposure Time (h/d × d/wk × mo)	Rat Tissue	Relative Adduct Labeling <sup>a</sup> (adducts/10 <sup>9</sup> nucleotides)			
				Control	Diesel Exhaust	Carbon Black	TiO <sub>2</sub>
Wong et al. 1986	Diesel exhaust (7.1 mg/m <sup>3</sup> )	5 × 5 × 7.5	Lung	1.0	21.0 <sup>b</sup>	ND	ND
Bond et al. 1988	Diesel exhaust (10 mg/m <sup>3</sup> )	7 × 5 × 3	Peripheral lung cells	5.0 ± 2.0	15.0 ± 2.0 <sup>b</sup>	ND	ND
Bond et al. 1990a	Diesel exhaust (0.35–10 mg/m <sup>3</sup> )	16 × 5 × 3	Peripheral lung cells	7.0 ± 3.0	14.0 ± 1.0 <sup>b,c</sup>	ND	ND
Bond et al. 1990b	Diesel exhaust (6.3 mg/m <sup>3</sup> ) Carbon black (6.3 mg/m <sup>3</sup> )	16 × 5 × 3	Alveolar type II cells	5.0 ± 2.0	23.0 ± 5.0 <sup>b</sup>	25.0 ± 2.0 <sup>b</sup>	ND
Wolff et al. 1990	Diesel exhaust (10 mg/m <sup>3</sup> ) Carbon black (10 mg/m <sup>3</sup> )	7 × 5 × 3	Lung	7.0 ± 3.0	17.0 ± 2.0 <sup>b</sup>	11.0 ± 2.0	ND
Mauderly et al. 1994	Diesel exhaust (6.5 mg/m <sup>3</sup> ) Carbon black (6.5 mg/m <sup>3</sup> )	16 × 5 × 3	Lung	38 ± 2.0	83 ± 7.0 <sup>b</sup>	38 ± 6.0	ND
Gallagher et al. 1994	Diesel exhaust <sup>d</sup> (7.5 mg/m <sup>3</sup> ) Carbon black (11.3 mg/m <sup>3</sup> ) TiO <sub>2</sub> (10.4 mg/m <sup>3</sup> )	16 × 5 × 24 <sup>d</sup>	Peripheral lung cells	10.0 ± 2.0 <sup>e</sup> 28.0 ± 3.1 <sup>f</sup>	16.0 ± 6.0 <sup>e</sup> 30.0 ± 1.0 <sup>f</sup>	13.0 ± 0.4 <sup>e</sup> 24.0 ± 2.0 <sup>f</sup>	13.0 ± 0.9 <sup>e</sup> 27.0 ± 4.0 <sup>f</sup>
Randerath et al. 1995	Diesel exhaust <sup>g</sup> (6.5 mg/m <sup>3</sup> ) Carbon black <sup>g</sup> (6.5 mg/m <sup>3</sup> )	16 × 5 × 24 <sup>g</sup>	Lung	28 ± 2.0 <sup>h</sup> 55 ± 4.0 <sup>i</sup>	42 ± 4.0 <sup>b,h</sup> 71 ± 5.0 <sup>i</sup>	34 ± 2.0 <sup>h</sup> 64 ± 5.0 <sup>i</sup>	ND ND

<sup>a</sup> Relative adduct labeling was calculated by dividing the counts per minute (cpm) detected in the adducted nucleotides by the cpm associated with a spectrophotometrically determined amount of labeled 2'-deoxyadenosine 3'-phosphate standard. The values were then converted into RAL after correcting for the dilution factor and volume spotted. RAL values are expressed as means ± SE. ND = not done.

<sup>b</sup> Statistically significant ( $p < 0.05$ ) compared with control values using Student's *t*-test, except in the studies by Randerath and coworkers (1995) and Mauderly and associates (1994), in which statistical significance was determined using Tukey's multiple comparison method.

<sup>c</sup> DNA adduct levels were similar at all concentrations.

<sup>d</sup> Adduct levels were also determined after 2 and 6 months.

<sup>e</sup> Nuclease P1 version of <sup>32</sup>P-postlabeling.

<sup>f</sup> Butanol extraction version of <sup>32</sup>P-postlabeling.

<sup>g</sup> Adduct levels were determined at 3, 6, 12, 18, and 23 months.

<sup>h</sup> Adduct levels at 3 months.

<sup>i</sup> Adduct levels at 24 months.

level of DNA adducts over control rats only at 3 months of exposure; however, they observed no correspondingly significant differences at later time points (6, 12, 18, and 23 months). The investigators observed many subtle quantitative changes (increases and reduction), however, in the levels of a specific class of adducts termed indigenous compounds (I-compounds) (Randerath et al. 1995). Such adducts are detected in unexposed animals and are assumed to arise indigenously in the absence of exposure to exogenous carcinogens; presumably they are formed when as-yet-unidentified endogenous electrophiles react with DNA (Randerath et al. 1986). The levels and profiles of these I-compounds are influenced by the test animal's age, hormonal status, diet (Randerath et al. 1990), and exposure to carcinogens (Randerath et al. 1992b,c). In rats exposed to diesel exhaust at 6.5 mg/m<sup>3</sup>, Randerath and colleagues (1995) found that most individual adducts (I-compounds) increased only at the 3-month time point and one adduct decreased at 23 months when compared with controls.

In the same study, Randerath and coworkers (1995) also investigated the time course of adduct formation in mouse skin painted with extracts of the same diesel exhaust particulate matter used in the animal bioassay (Table 3). In the skin, total adduct levels peaked at day one and rapidly declined after three days. In the lung, one major adduct and several minor adducts were observed and their levels remained steady for six weeks. The pattern of adducts was similar regardless of whether extracts were administered in a single or in multiple applications; however, the pattern was different from that observed when the skin of mice was painted with cigarette smoke condensates (Randerath et al. 1992a). This variance suggests the presence of different types of chemicals in these two complex mixtures.

### Specific Diesel Exhaust Adducts

In most studies, investigators have been unable to identify specific adducts induced by diesel exhaust (Wong et al. 1986; Bond et al. 1988, 1990a,b; Wolff et al. 1990; Mauderly et al. 1994; Randerath et al. 1995). Only Gallagher and colleagues (1994) reported identifying a DNA adduct specific to diesel exhaust, which they attributed to a nitro-PAH because it was detected only in the nucleotides analyzed by butanol extraction and not by the P1 nuclease version of the <sup>32</sup>P-postlabeling assay, a procedure claimed by the investigators to underrepresent nitro-PAHs. This may not be generally true, however, because certain nitro-PAH-DNA adducts have been detected by the nuclease P1 procedure (Roy et al. 1989; Delcos et al. 1993; El-Bayoumy et al. 1994). Further, the investigators reported that the chromatographic properties of this adduct were similar to those of nitrobenzo[*a*]pyrene and nitrochrysene. The same or a related adduct,

however, was also detected in the filtered air controls at the two-month time point, and no attempt was made to chromatographically characterize the adduct induced by diesel exhaust by comparison with authentic nitro-PAH-derived markers. Additionally, Gallagher and coworkers (1994) did not detect any PAH-derived DNA adducts in the lungs of rats exposed to diesel exhaust.

In another study (Gallagher et al. 1990), mouse skin was painted with particulate extracts from either coke oven smoke, coal soot, or diesel exhaust. The observed DNA adduct patterns differed according to sample type; however, one adduct that was common to all the three samples comigrated with the major benzo[*a*]pyrene (BaP) DNA adduct. The investigators concluded that, on the basis of the relative concentration of BaP in each of the mixtures (0.5 g for diesel exhaust, 19.7 g for coal soot, and 35 g for coke oven smoke), it was unlikely that this adduct, which represented approximately 12% to 34% of the total number of adducts for the coke oven smoke and coal soot samples and 49% to 67% for the diesel exhaust sample, was derived from BaP alone. The highest total number of adducts resulted from painting mouse skin with coke oven smoke extract followed by coal soot and diesel exhaust (Table 3).

### Route of Administration

Compared with inhalation studies, higher levels of DNA adducts were reported in skin-painting experiments using organic solvent extracts of diesel exhaust particles (Table 3; Gallagher et al. 1990 and Randerath et al. 1995). This difference may reflect species variability, given that all skin-painting experiments were performed in mice; more likely, however, the difference is attributable to the method of extract application. In the skin-painting experiments, test animals were exposed to a multitude of DNA-reactive chemicals extracted in organic solvent, whereas in the inhalation experiments the rats were exposed to bound chemicals, which may not have been readily bioavailable. Other factors that may have played a role include route of administration, dose over time, and chronic versus acute exposures.

### Carbon Black

Two recent studies (Heinrich 1994; Mauderly et al. 1994) reported similar rates of carcinogenicity in rats exposed to whole diesel exhaust, to particles devoid of the adsorbed polycyclic organic material (i.e., carbon black), or to TiO<sub>2</sub> particles (discussed in detail in the background paper by Busby and Newberne, this report). In some studies designed to investigate genotoxicity after exposure to such particles, total DNA adduct levels in rats exposed to carbon black alone were generally at an intermediate value between the levels in controls and levels in those treated with complete

diesel exhaust (Wolff et al. 1990). Bond and coworkers (1990b) showed that exposure to both types of particles induced significantly higher DNA adduct levels than in control animals. At three months, animals treated with carbon black had developed lower DNA adduct levels than animals treated with diesel exhaust (Mauderly et al. 1994; Randerath et al. 1995). Gallagher and coworkers (1994), however, reported that inhalation of poorly soluble, nonfibrous particles such as carbon black and TiO<sub>2</sub> had no effect on DNA adduct levels in Wistar rats after two years. Both Gallagher and coworkers (1994) and Mauderly and coworkers (1994) conducted DNA adduct studies on the tissues of animals with very high particle lung burdens, inflammation, extensive epithelial cell proliferation, and fibrosis. In their study, Wolff and colleagues (1990) also reported a mild inflammatory response, changes in bronchoalveolar lavage parameters, and the presence of particle-laden macrophages. Reactive oxygen species released by phagocytes (macrophages and neutrophils) are known to be genotoxic and cause other forms of cell injury via lipid peroxidation (Frenkel 1992). Oxygen-free-radical-mediated induction of DNA strand breaks, DNA-protein crosslinks, and lipid peroxidation can induce the growth and differentiation of protooncogenes and tumor suppressor genes (Cerutti and Trump 1991). It is possible that a high lung burden of particles

(with or without adsorbed PAHs) induced the DNA adducts via the release of oxygen free radicals by macrophages; however, these types of oxidative DNA lesions may not have been observed because their detection would require chromatographic conditions different from those employed in the above studies (Marnett and Burcham 1993).

### Tumors

Bond and colleagues (1988) investigated the possible relationship between DNA adducts and tumor formation by determining whether type II cells, the cells thought to be at risk from diesel exhaust exposure, are particularly susceptible to DNA adduct formation as a consequence of exposure to diesel exhaust. Indeed, elevated levels of DNA adducts were found in tissue taken from the same (peripheral) region of the lung where tumors had developed in rats exposed to high concentrations of diesel exhaust (Mauderly et al. 1987), suggesting a link between DNA adducts and subsequent tumor formation.

Other investigators have offered suggestions on the possible role of I-compounds in inducing carcinogenicity (Randerath et al. 1993; Gallagher et al. 1994). Randerath and coworkers (1995) concluded (1) that because both carbon black and diesel exhaust particles induced DNA adducts, the forma-

**Table 3.** Summary of DNA Adduct Studies: Skin Painting with Extracts of Diesel Exhaust and Other Particulate Matter

Reference	Source of Particulate Matter Extract	Dose per Mouse	Tissue	Control Group	Relative Adduct Labeling <sup>a</sup> (adducts/10 <sup>9</sup> nucleotides)		
					Diesel Exhaust	Coal Soot	Coke Oven Smoke
Gallagher et al. 1990	Diesel exhaust, coal soot, coke oven smoke	20 mg	Skin Lung	50 ± 5.0 29 ± 1.0	129 ± 22 <sup>b</sup> 83 ± 17 <sup>b</sup>	750 ± 160 <sup>b</sup> 320 ± 50 <sup>b</sup>	1,445 ± 580 <sup>b</sup> 1,040 ± 240 <sup>b</sup>
Randerath et al. 1995	Diesel exhaust	20 mg × 1 20 mg × 5	Skin Lung Skin Lung	ND	121 ± 12 <sup>c</sup> ; 63 ± 8.0 <sup>d</sup> 9.0 ± 2.0 <sup>c</sup> ; 12 ± 2.0 <sup>d</sup> 188 ± 2.2 <sup>e</sup> ; 36 ± 0.4 <sup>f</sup> 27 ± 3.0 <sup>e</sup> ; 30 ± 5.0 <sup>f</sup>	ND	ND

<sup>a</sup> Relative adduct labeling was calculated by dividing the cpm detected in the adducted nucleotides by the cpm associated with a spectrophotometrically determined amount of labeled 2'-deoxyadenosine 3'-phosphate standard. The values were then converted into RAL after correcting for the dilution factor and volume spotted. RAL values are expressed as means ± SD. ND = not done.

<sup>b</sup> Statistically significant compared with control values using Student's *t*-test.

<sup>c</sup> One dose; adduct levels after one day.

<sup>d</sup> One dose; adduct levels after three days.

<sup>e</sup> Five doses; adduct levels after one day.

<sup>f</sup> Five doses; adduct levels after three days.

tion of adducts was not related to the organic content of the particles, and (2) that the observed changes in the levels of I-compounds may have been responsible for the observed carcinogenicity of diesel exhaust and carbon black (Mauderly et al. 1994). Gallagher and colleagues (1994) detected one type of adduct (I-compound) that increased significantly over time in the control group exposed to filtered air, but decreased markedly at the two-year time point in animals treated with diesel exhaust, carbon black, and TiO<sub>2</sub>. This finding suggests that the adduct was endogenous rather than exogenous. The investigators suggested that either the reduction in the levels of I-compounds may reflect adduct dilution as a result of de novo cell synthesis induced by the high particle lung burden, or these exposures affected the endogenous synthesis or degradation of DNA-reactive precursors. Indeed, as discussed earlier, Randerath and coworkers (1995) observed an increase in the levels of certain I-compounds and a decrease in the level of one I-compound as a result of diesel exhaust exposure.

Investigators have reported decreased levels of I-compounds in the liver, kidney, and lung tissue of strains of mice that have a high susceptibility to tumors in these organs (Randerath et al. 1986; Li and Randerath 1990) and decreased levels in response to cytochrome P450 inducers (Moorthy et al. 1994). Randerath and coworkers (1993) suggest that these DNA modifications may represent biomarkers of aging, nutritional status, tumor promotion, and carcinogenesis. Because the mechanism or mechanisms of these effects remain essentially undetermined, it is unclear whether modulation of I-compound levels may be an important factor in the tumorigenic response produced by both diesel exhaust and poorly soluble particles.

#### MUTAGENIC COMPOUNDS IN DIESEL EMISSIONS

Because diesel exhaust is a complex mixture containing thousands of chemicals, identifying the most biologically active compounds presents an enormous, if not impossible, task. In the mid-1970s Huisingh and associates (1978) used short-term bioassays in conjunction with analytical chemistry measurements to identify mutagens from such complex mixtures and coined the term bioassay-directed chemical analysis. In most cases, the toxicologically relevant PAHs in diesel particulate matter are determined by extracting the diesel particulate fraction with organic solvents and testing the fractions for mutagenicity, and then performing fractionation by high-performance liquid chromatography or gas chromatography coupled with mass spectrometry and bioassays. Numerous detection schemes have been reported in the literature (Levsen et al. 1987).

Many of the chemicals detected in diesel exhaust have demonstrated mutagenic properties in a variety of short-term tests (International Agency for Research on Cancer 1983, 1989), as discussed below. Table 4 (adapted from Scheepers and Bos 1992b) lists chemicals identified in diesel exhaust that have been classified as carcinogens in animals by the International Agency for Research on Cancer (1983, 1989); Table 4 also provides the results of mutagenicity studies of these chemicals using bacterial or mammalian mutation assays as reported by the International Agency for Research on Cancer (1983, 1987, 1989).

#### PARTICLE-ASSOCIATED ORGANIC COMPOUNDS

##### Polycyclic Aromatic Hydrocarbons

A number of indirect-acting PAHs (Table 4), most notably BaP, a mutagen and carcinogen (International Agency for Research on Cancer 1987), have been identified in diesel exhaust. To date, the clearest demonstration that extracts of diesel exhaust particles contain indirect-acting mutagens has come from experiments with *S. typhimurium* strains (TA 98NR, TA 98DNP<sub>6</sub>) that are resistant to mutation by nitro-PAHs (Pederson and Siak 1981). With these strains the direct-acting mutagenicity of the extracts was attenuated sufficiently that activation by mixing with S9 could be detected. Ball and colleagues (1990) separated diesel particle extracts into eight fractions using high-performance liquid chromatography and subjected each to the *Salmonella* mutagenicity assay. For the fraction containing the classic PAHs (e.g., fractions containing BaP), the S9-activated mutagenic activity was essentially undetectable, even though the fraction constituted 5% of the extract mass. A fraction that eluted between the classic PAH fraction and the nitro-PAH-containing fraction was weakly active without the S9 mix, but was 20 times more active with S9. This indirect activity, although easily detectable in the chromatographic fraction, was too weak to be detected in unfractionated extracts because it was obscured by the direct-acting activity of the other components. Chemicals that corresponded to prominent peaks in the gas chromatograms (i.e., those in the fraction that showed strong mutagenicity in the presence of S9) were tentatively identified to be monomethyl- and dimethylnitroanthracenes or phenanthrenes on the basis of their mass spectra. Although the mutagenicity of these chemicals has not been determined, similar chemicals, such as 2- and 9-nitroanthracene, are weakly mutagenic with S9 activation (Fu et al. 1985).

Using the <sup>32</sup>P-postlabeling assay, DNA adducts have been demonstrated for BaP, benzo[e]pyrene, chrysene, benz[a]anthracene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, and

benzo[*g,h,i*]perylene, but not for pyrene, anthracene, and perylene (Reddy and Randerath 1984). In an investigation on the formation and persistence of BaP-DNA adducts in rat lung, liver, and peripheral blood lymphocytes using the nuclease P1 version of the <sup>32</sup>P-postlabeling assay, lung DNA was found to contain maximal levels of BaP-induced DNA adducts after intraperitoneal injection (Ross et al. 1990). Benzo[*b*]fluoranthene was also found to induce persistent

DNA adducts in rat lung, liver, and peripheral blood lymphocytes (Nesnow et al. 1993). Benzo[*b*]fluoranthene and benzo[*j*]fluoranthene produced adducts in mouse skin (Weyand et al. 1993a,b). Other PAHs present in diesel exhaust that were found to induce DNA adducts in rat lungs after being instilled intratracheally are benz[*a*]anthracene and dibenz[*a,h*]anthracene (Whong et al. 1994).

**Table 4.** Mutagenic Chemicals in Diesel Exhaust That Have Been Evaluated for Carcinogenicity by the International Agency for Research on Cancer<sup>a</sup>

Compound	Mutagenicity		Evidence for Carcinogenicity in Animals	Compound	Mutagenicity		Evidence for Carcinogenicity in Animals
	<i>S. typhimurium</i>	Mam-malian			<i>S. typhimurium</i>	Mam-malian	
<b>Olefins<sup>b</sup></b>				Chrysene	+P	-	Limited
1,3-Butadiene	+	+	Sufficient	Coronene	+P	NA	Inadequate
Ethylene <sup>c</sup>	-	+	Inadequate	Cyclopenta[ <i>c,d</i> ]pyrene	+P	+P	Limited
Propylene <sup>c</sup>	+	+	Inadequate	Dibenz[ <i>a,h</i> ]anthracene	+P	+	Sufficient
<b>Aromatic<sup>b</sup></b>				Fluoranthene	+P	+P	No evidence
Benzene	-	+	Sufficient	Fluorene	-	NA	Inadequate
<b>Aldehydes</b>				Indeno[1,2,3- <i>c,d</i> ]pyrene	+P	+	Sufficient
Acetaldehyde	+	+	Sufficient	Perylene	+P	-	Inadequate
Acrolein	+	NA <sup>d</sup>	Inadequate	Phenanthrene	+	-	Inadequate
Formaldehyde	+	+	Sufficient	Pyrene	+P	NA	No evidence
<b>PAHs<sup>e</sup></b>				Triphenylene	+P	NA	Inadequate
Anthanthrene	+P <sup>f</sup>	NA	Limited	<b>Methyl-PAHs</b>			
Anthracene	-	-	No evidence	3-Methylchrysene	+	+	Limited
Benz[ <i>a</i> ]anthracene	+P	+P	Sufficient	1-Methylphenanthrene	+	+	Inadequate
Benzo[ <i>b</i> ]fluoranthene	+P	NA	Sufficient	<b>Nitro-PAHs<sup>g</sup></b>			
Benzo[ <i>j</i> ]fluoranthene	+P	NA	Sufficient	3,7-Dinitrofluoranthene	+	+	Limited
Benzo[ <i>k</i> ]fluoranthene	+P	NA	Sufficient	3,9-Dinitrofluoranthene	+	+	Limited
Benzo[ <i>g,h,i</i> ]fluoranthene	+P	NA	Inadequate	1,3-Dinitropyrene	+	+	Limited
Benzo[ <i>a</i> ]fluorene	-	NA	Inadequate	1,6-Dinitropyrene	+	+	Sufficient
Benzo[ <i>b</i> ]fluorene	-	NA	Inadequate	1,8-Dinitropyrene	+	+	Sufficient
Benzo[ <i>g,h,i</i> ]perylene	+P	-	Inadequate	7-Nitrobenz[ <i>a</i> ]anthracene	-	NA	Limited
Benzo[ <i>a</i> ]pyrene	+P	+P	Sufficient	6-Nitrobenzo[ <i>a</i> ]pyrene	+P	+/-P	Limited
Benzo[ <i>e</i> ]pyrene	+P	-	Inadequate	2-Nitrofluorene	+	+	Sufficient
				1-Nitropyrene	+	+	Sufficient

<sup>a</sup> Data are from Scheepers and Bos (1992b) unless otherwise noted.

<sup>b</sup> Data are from International Agency for Research on Cancer (1987).

<sup>c</sup> Alkylating intermediates ethylene oxide and propylene oxide are mutagenic.

<sup>d</sup> NA = Data not available.

<sup>e</sup> Data are from International Agency for Research on Cancer (1983).

<sup>f</sup> P = in the presence of metabolic activation.

<sup>g</sup> Data are from International Agency for Research on Cancer (1989).

### Alkylated Polycyclic Aromatic Hydrocarbons

Some alkylated PAHs may be as strong as or even more mutagenic and carcinogenic than the parent PAHs (Glatt et al 1990). Barfknecht and coworkers (1982) found that diesel particle extracts demonstrated mutagenic activity when tested with human lymphoblasts; however, this activity was detected only in the presence of S9. Of the 11 PAHs identified in the extract, 4 PAHs (fluoranthene, 1-methylphenanthrene, 9-methylphenanthrene, and BaP) accounted for approximately 45% of the total extract activity. These results suggest a possible contribution of alkylated PAHs to the mutagenicity of diesel exhaust extracts. Dimethylfluorene and trimethylfluorene have been shown to be mutagenic after metabolic activation (LaVoie et al. 1985). Yu and Hites (1981) suggested that methylphenanthrenes and methylfluorenes may be more important mutagens than BaP in diesel exhaust.

Methyl groups substituted in the nonbenzo-ring bay region of some PAHs showed enhanced mutagenicity in the human hepatoma cell mediated assay (Diamond et al. 1984). 1-Methylpyrene and 1,6-dimethylpyrene, which are also present in diesel exhaust, induced unscheduled DNA synthesis in rat hepatocytes, and 1-methylpyrene was carcinogenic in the newborn mouse assay (Rice et al. 1987).

### Nitro-Polycyclic Aromatic Hydrocarbons

The direct-acting mutagenicity of diesel particulate extracts has been attributed to nitro-PAHs, which have been identified as major mutagens in extracts of diesel particulate matter. Attempts to quantify the contribution of the different chemicals identified in diesel particulate extracts to the mutagenic activity of the crude extract have met with mixed success. This is partially because different investigators have used different samples of diesel exhaust and also different experimental methods. The fraction of direct-acting mutagenicity attributed to 1,3-, 1,6-, and 1,8-dinitropyrenes ranged from approximately 20% to 40% (Nakagawa et al. 1983; Manabe et al. 1985). The contribution of activity attributed to 1-nitropyrene has been estimated at approximately 4% to 12% (Rosenkranz et al. 1982; Nakagawa et al. 1983; Saleem et al. 1984; Manabe et al. 1985). The general importance of nitro-PAHs to the direct-acting mutagenicity of particle extracts is also indicated by data from *Salmonella* mutagenicity assays using nitroreductase and *o*-transacetylase-deficient strains TA 98NR and TA 98DNP<sub>6</sub>. For various extracts, the activities observed with strain TA 98NR were 50% to 70% of those observed with TA 98, and those observed with TA 98DNP<sub>6</sub> were 30% to 50% (Brooks et al. 1980; Pederson and Siak 1981; Saleem et al. 1984; Manabe et al. 1985), suggesting that nitro-PAHs accounted for a majority of the mutagenicity. Direct-acting

mutagens identified in diesel particle extracts also include hydroxynitropyrenes and acetoxynitropyrenes (Pederson and Siak 1981; Saleem et al. 1982, 1984; Manabe et al. 1985; Raat 1988); however, the carcinogenicity of these compounds has not been confirmed.

Of the nitro-PAHs that have been classified as carcinogens, 10 have been reported to be constituents of diesel exhaust (Paputa-Peck et al. 1983; Tokiwa et al. 1986; MacCrehan et al. 1988). The nitro-PAHs classified as carcinogens based on animal studies include 2-nitrofluorene, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, and 1,8-dinitropyrene (Table 4). Hecht and El-Bayoumy (1991) have suggested that the nitro-PAHs can be more potent tumorigens than PAHs (e.g., BaP) and that several nitro-PAHs (6-nitrochrysene and 1,6-dinitropyrene) are potent rodent respiratory carcinogens. Among the nitro-PAHs present in diesel exhaust (Table 4), mainly 1-nitropyrene and 1,6-dinitropyrene induce DNA adducts *in vivo* in rat mammary glands after intraperitoneal injection (Beland 1989, 1991; Smith et al. 1990); also, 1-nitropyrene has been found to induce DNA adducts in the mammary and liver tissue of rats treated orally (Roy et al. 1989; El-Bayoumy et al. 1994). A major 1,6-dinitropyrene-DNA adduct was detected in the lung and spleen lymphocytes of rats after intratracheal instillation (Smith et al. 1993; Beland et al. 1994). The same adduct was found in rat mammary glands and nucleated blood cells (Djuric et al. 1993), and it was also observed in rat liver, mammary glands, and peripheral lymphocytes after oral administration (El-Bayoumy et al. 1994).

Although nitrated-PAHs have been shown to account for a major portion of the mutagenic activity associated with diesel emissions (Schuetzle et al. 1981), it is not known whether or to what extent nitrated-PAHs present in diesel emissions contributed to the observed carcinogenicity in rats (see Part II of this background paper, by Rosenkranz). It should be noted that the contribution of nitro-PAHs to the diesel exhaust particles on a weight basis is much lower than that of the parent PAHs (Raat 1988). Moreover, lung implantation studies of fractions of diesel exhaust extracts containing nitro-PAHs revealed only relatively low tumor incidence (Grimmer et al. 1987). Recently, 1,6-dinitropyrene, a potent carcinogen present in diesel exhaust, has been shown to be a strong *in vivo* mutagen when instilled intratracheally in rats (Smith et al. 1993; Beland et al. 1994) under conditions shown to result in 1,6-dinitropyrene-induced lung tumors (Iwagawa et al. 1989). Beland and coworkers (1994), however, calculated that under conditions of diesel inhalation bioassays (Mauderly et al. 1987, 1994), the level of 1,6-dinitropyrene was insufficient to account for the observed carcinogenicity, at least in the rat lung.

In a recent study by Lee and coworkers (1994), BaP was found to inhibit the bacterial mutagenicity and DNA adduct-forming ability of 1-nitropyrene by altering its nitroreductive metabolism. These findings suggest that factors such as chemical interactions and modification of metabolism between and among the constituents present in complex mixtures influence the contribution of genotoxic chemicals to the observed carcinogenicity of such mixtures.

#### Oxygenated Polycyclic Aromatic Hydrocarbons

A majority of the mutagenic activity of diesel engine exhaust extracts is attributable to a fraction that is composed of oxygenated PAH species (Schuetzle et al. 1981; Schuetzle 1983; Ciccioli et al. 1986; Williams et al. 1986). These derivatives include hydroxy, dihydroxy, aldehyde, anhydride, quinone, and ketone groups. Some of these compounds are unstable during isolation (Schuetzle et al. 1981). Ball and Young (1992) found that a fraction of diesel particle extracts showed direct-acting mutagenicity in the *Salmonella* tester strain TA 102. This strain is sensitive to oxidative mutagens (Levin et al. 1982) (i.e., classes of compounds that can participate in redox reactions, such as quinones, or generate free radicals, such as hydroperoxides). The investigators also found that the mutagenicity of several selected nitro-PAHs was significantly reduced in TA 102 compared with TA 98 and that only 1% of the total TA 102 mutagenicity could be accounted for by these selected nitro-PAHs. The investigators suggested that activity induced by TA 102 could be attributable to quinones (e.g., pyrene quinone), which have been identified in diesel particle extracts (Schuetzle et al. 1985). The significance of quinones with respect to the toxicity of diesel exhaust is still unclear; however, it should be noted that the reduced response observed in the study by Ball and Young (1992) may have been due to such factors as the detoxification of mutagens or nonspecific binding of compounds by lipoproteins, or to the detoxification of some compounds and activation of others, or simply to a different target DNA sequence (McCoy et al. 1985).

#### Heterocyclic Polycyclic Aromatic Hydrocarbons

Many sulfur analogues of PAHs, such as thiaarenes, have been identified in diesel exhaust (Schuetzle et al. 1981, 1985; Ciccioli et al. 1986; Davies et al. 1988; Jacob 1990; Scheepers and Bos 1992b). No attempts have been made to calculate the contribution of thiaarenes to the total mutagenicity of diesel exhaust. Although the carcinogenic effects of thiaarenes have not been studied extensively, several four- and five-ring compounds have been reported to possess carcinogenic potential (reviewed by Jacob 1990).

Other heterocyclic PAHs, such as nitroquinolines, have been identified in diesel exhaust extracts by Schuetzle and colleagues (1985).

#### VAPOR-PHASE COMPOUNDS

Although many studies focusing on diesel particles have been conducted, little information is available on the vapor-phase emissions of diesel exhaust. Chemicals present in the vapor phase of diesel exhaust consist of unburned or partially oxidized hydrocarbons that have a low molecular weight and remain in the vapor phase even after mixing with cool ambient air. These volatile compounds cannot adsorb or condense onto the particles and are not collected by conventional sampling procedures.

The vapor-phase exhaust, which has been described in great detail elsewhere (International Agency for Research on Cancer 1989), contains a variety of carcinogenic and mutagenic compounds (Schuetzle 1983; Dorie et al. 1987; Coutant et al. 1988; International Agency for Research on Cancer 1989). Gaseous emissions from diesel exhaust collected by condensation after dilution and filtration of particles were found to be mutagenic to *S. typhimurium* in both the presence and absence of an S9 mixture (in Table 1 see Rannug et al. 1983 and Matsushita et al. 1986), indicating that the mutagenicity is direct-acting. These studies differ in the quantitative estimates of the contribution that gaseous emissions make to the total mutagenicity of diesel exhaust: direct testing of gaseous emissions suggests that the gas phase contributes at least 30 times more to the total mutagenicity than the particulate phase (Matsushita et al. 1986); testing of the condensation extract indicated that the gaseous emissions contribute less than the particulate phase to total mutagenicity (Rannug et al. 1983). Stump and coworkers (1982) found that the vapor-phase mutagenicity was approximately 10% of total mutagenicity in particle extracts using light-duty diesel engine exhaust. Bagley and coworkers (1987, 1993) found that the vapor phase contributed up to 25% of the direct-acting mutagenicity (per cubic meter of raw exhaust) associated with heavy-duty diesel engine exhaust. Both Stump and associates (1982) and Bagley and coworkers (1987, 1993) assayed extracts of semi-volatile organic compounds collected on an XAD resin, and the samples exhibited primarily direct-acting mutagenicity.

A considerable amount of ethylene has been found in the gas phase. This compound may contribute to the genotoxic properties of the gas phase, given that it is metabolized to alkylating intermediates of ethylene oxide in mammalian cells (Tornquist et al. 1986). Other mutagenic and carcinogenic compounds present in the vapor phase include ben-

zene, 1,3-butadiene, acetaldehyde, acrolein, formaldehyde, and a variety of PAHs and their derivatives (International Agency for Research on Cancer 1989).

## SUMMARY

Diesel exhaust particles or their extracts have been studied in a variety of genotoxicity bioassays. Indeed, the extracts have been more widely tested than whole diesel exhaust in bacterial and mammalian mutation assays. In general, results in these bioassays have been positive. Unlike the bacterial assays, the mammalian assays have been active only in the presence of metabolic activation, suggesting that the bacterial assays detect the direct-acting mutagens (e.g., PAH derivatives, nitro-PAHs), whereas the mammalian assays are sensitive to the parent PAHs. Exhaust particles or their extracts also induce chromosome aberrations, SCE, micronuclei formation, and cell transformation in mammalian and human cells in culture. In general, whole diesel exhaust yielded negative results in *in vivo* tests for SCE, and results in the micronucleus test were mixed. Results in some of the studies on DNA adducts showed that exposure of rats by inhalation to diesel exhaust induced a small but significant increase in total DNA adduct levels when compared with controls. Randerath and coworkers (1995) reported an increase in total adduct levels in diesel exhaust-exposed animals at three months but not at later time points. Gallagher and coworkers (1994), however, did not observe any change in total DNA adduct levels over controls exposed to filtered air in studies with rats that inhaled poorly soluble nonfibrous particles such as carbon black and TiO<sub>2</sub>. In general, none of the inhalation studies reported the presence of diesel exhaust-specific DNA adducts. Painting the skin of animals with extracts of diesel particles, however, induced high levels of DNA adducts.

Results of mutation bioassays performed in conjunction with chemical analysis showed that nitro-PAHs are the principal mutagenic species in diesel exhaust, accounting for approximately 50% of the mutagenicity of diesel exhaust extracts. Other PAH derivatives with strong mutagenic potencies include methyl PAHs and oxygenated PAHs; however, the contribution of these organic compounds to the carcinogenicity of diesel exhaust is not known. The gas phase contains a number of potent mutagens and carcinogens that may contribute to the overall mutagenicity and carcinogenicity of diesel exhaust.

It is clear from the results of mutagenicity and genotoxicity bioassays that diesel exhaust particles and the adsorbed organic compounds are genotoxic. Many of the organic compounds (PAHs and their derivatives) are known to

induce DNA adducts in animals *in vivo*. Because most of the studies failed to detect diesel exhaust-specific DNA adducts when rats inhaled a high concentration of particles (with or without adsorbed organic compounds), the investigators (Gallagher et al. 1994; Mauderly et al. 1994; Randerath et al. 1995) concluded that the particle-associated organic compounds do not elicit DNA adduct formation, as detected by the nuclease P1 or butanol extraction version of the <sup>32</sup>P-postlabeling assay. It is conceivable, however, that oxidative DNA adducts were formed as a result of the inflammatory process associated with high particle accumulation in the lung, but that these types of oxidative lesions were not detected by the analytical methods used in the studies. The role of genotoxic chemicals in diesel exhaust-induced carcinogenesis is not clear. Several analyses (Rosenkranz 1993; Beland et al. 1994; Heinrich 1994; Mauderly 1994) suggest that the carcinogenic effect elicited by diesel exhaust in rats may not be primarily due to mutagenicity or genotoxicity of the organic chemicals adsorbed onto the diesel particles.

## REFERENCES

- Bagley ST, Gratz LD, Leddy DG, Johnson JH. 1993. Characterization of particle- and vapor-phase organic fraction emissions from a heavy-duty diesel engine equipped with a particle trap and regeneration controls. Research Report No. 56. Health Effects Institute, Cambridge, MA.
- Bagley ST, Korie LD, Leddy LC, Johnson JH. 1987. An investigation into the effects of ceramic particle traps on the chemical mutagens in diesel exhaust. Research Report No. 5. Health Effects Institute, Cambridge, MA.
- Ball JC, Barrie G, Young WC, Richeri JFO, Saleem IT. 1990. S9 mediated Ames assay of diesel particle extracts detecting indirect-acting mutagens in samples that are direct-acting. *Environ Sci Technol* 24:890-894.
- Ball JC, Young WC. 1992. Evidence for a new class of mutagens in diesel particulate extracts. *Environ Sci Technol* 26:2181-2186.
- Barfknecht TR, Hites RA, Cavaliers EL, Thilly WG. 1982. Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. In: *Toxicological Effects of Emissions from Diesel Engines* (Lewtas J, ed.) pp. 277-294. Elsevier Science Publishing Co., New York, NY.
- Beland FA. 1989. DNA binding by 1-nitropyrene and dinitropyrene *in vitro* and *in vivo*: Effects of nitroreductase induction. Research Report No. 31. Health Effects Institute, Cambridge, MA.

- Beland FA. 1991. Role of ring oxidation in the metabolic activation of 1-nitropyrene. Research Report No. 46. Health Effects Institute, Cambridge, MA.
- Beland FA, Fullerton NF, Smith BA, Heflich RH. 1994. Formation of DNA adducts and induction of mutations in rats treated with tumorigenic doses of 1,6-dinitropyrene. *Environ Health Perspect* 102:185-189.
- Belisario MA, Buonocore V, De Martinis E, De Lorenzo F. 1984. Biological availability of mutagenic compounds adsorbed onto diesel exhaust particulate. *Mutat Res* 135:1-9.
- Belisario MA, Farina C, Buonocore V. 1985. Evaluation of concentration procedures of mutagenic metabolites from urine of diesel particulate-treated rats. *Toxicol Lett* 25:81-83.
- Bond JA, Johnson NF, Snipes MB, Mauderly JL. 1990a. DNA adduct formation in rat alveolar type II cells: Cells potentially at risk for inhaled diesel exhaust. *Environ Mol Mutagen* 16:64-69.
- Bond JA, Mauderly JL, Wolff RK. 1990b. Concentration- and time-dependent formation of DNA adducts in lungs of rats exposed to diesel exhaust. *Toxicology* 60:127-135.
- Bond JA, Wolff RK, Harkema JR, Mauderly JL, Henderson RF, Griffith WC, McClellan RO. 1988. Distribution of DNA adducts in the respiratory tracts of rats exposed to diesel exhaust. *Toxicol Appl Pharmacol* 96:336-346.
- Brooks AL, Wolff RK, Royer RE, Clark CR, Sanchez A, McClellan RO. 1980. Biological availability of mutagenic chemicals associated with diesel exhaust particles. In: *Health Effects of Diesel Engine Emissions* (Pepelko WE, Danner RM, Clarke NA, eds.) pp. 345-358. EPA/600/9-80/57a. U.S. Environmental Protection Agency, Cincinnati, OH.
- Casto BC, Hatch GC, Huang SL. 1981. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: In vitro mutagenesis. *Environ Int* 5:403-409.
- Cerutti PA, Trump BF. 1991. Inflammation and oxidative stress in carcinogenesis. *Cancer Cells* 1991:1-7.
- Chescheir GM, Garrett NE, Shelburne JD, Huisingsh JL, Waters MD. 1981. Mutagenic effects of environmental particulates in the CHO/HGPRT system. In: *Short-Term Bioassay in the Analysis of Complex Environmental Mixtures, II* (Nesnow S, ed.) pp. 337-350. Plenum Press, New York, NY.
- Ciccioli P, Brancaleoni E, Cecinato A, Dipalo C, Butinin P, Libert A. 1986. Fractionation of polar polynuclear aromatic hydrocarbons present in industrial emissions and atmospheric samples and their determination by gas chromatography-mass spectrometry. *J Chromatogr* 351:451-464.
- Clark CR, Vigil CL. 1980. Influence of rat lung and liver homogenates on the mutagenicity of diesel exhaust particulate extracts. *Toxicol Appl Pharmacol* 56:110-115.
- Coutant RW, Brown L, Chuang JC, Riggan RM, Lewis RG. 1988. Phase distribution and artifact formation in ambient air sampling for polynuclear aromatic hydrocarbons. *Atmos Environ* 72:403-409.
- Crebelli R, Fuselli S, Conti G, Carere A. 1991. Mutagenicity spectra in bacterial strains of airborne and engine exhaust particulate extracts. *Mutat Res* 261:237-248.
- Curren RD, Kouri RE, Kim CM, Schnectman LM. 1981. Mutagenic and carcinogenic potency of extracts from diesel related environmental emissions: Simultaneous morphological transformation and mutagenesis in BALB/C 3T3 cells. *Environ Int* 5:411-415.
- Davies IL, Bartle KD, Williams PT, Andrews GE. 1988. On-line fractionation and identification of diesel fuel polycyclic aromatic compounds by two-dimensional microbore high performance liquid chromatography. *Anal Chem* 60:204-209.
- Delcos KB, Manjanatha MG, Li EE, Newton RK, Mittlstaed RA, Heflich RH. 1993. <sup>32</sup>P-postlabelling studies of arylamine and nitroaromatic hydrocarbon activation and mutagenesis. In: *Postlabelling Methods for Detection of DNA Adducts*. International Agency for Research on Cancer Scientific Publication No. 124 (Phillips DH, Castegnaro M, Bartsch H, eds.) pp. 79-86. International Agency for Research on Cancer, Lyon, France.
- dell'Omo M, Lauwerys RR. 1993. Adducts to macromolecules in the biological monitoring of workers exposed to polycyclic aromatic hydrocarbons. *Crit Rev Toxicol* 23:111-126.
- Diamond L, Cherian K, Harvey RG, Digiovanni J. 1984. Mutagenic activity of methyl and fluoro substituted derivatives of polycyclic aromatic hydrocarbons in human hepatoma (HepG2) cell mediated assay. *Mutat Res* 136:65-72.
- Djuric Z, Potter DW, Culp SJ, Luongo DA, Beland FA. 1993. Formation of DNA adducts and oxidative DNA damage in rats treated with 1,6-dinitropyrene. *Cancer Lett* 71:51-56.
- Dorie LD, Bagley ST, Leddy DCT, Johnson JH. 1987. Identification of polycyclic aromatic hydrocarbons in mutagenic subfraction of diesel exhaust modified by a ceramic particulate trap. *Environ Sci Technol* 21:751-765.

- El-Bayoumy K, Johnson BE, Roy AK, Upadhaya P, Partian SJ. 1994. Biomonitoring of nitropolynuclear aromatic hydrocarbons via protein and DNA adducts. Research Report No. 64. Health Effects Institute, Cambridge, MA.
- Fredga K, Davring L, Sunner M, Bengtsson BO, Elinder CG, Sigtryggsson P, Berlin M. 1982. Chromosome changes in workers (smokers and nonsmokers) exposed to automobile fuels and exhaust gases. *Scand J Environ Health* 8:209-221.
- Frenkel K. 1992. Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacol Ther* 53:127-166.
- Fu PP, Heflich RH, Von Tungeln LS, Yang DT, Fifer EK, Beland FA. 1985. Effect of the nitro group conformation on the rat liver microsomal metabolism and bacterial mutagenicity of 2 and 9 nitroanthracene. *Carcinogenesis* 7:1819-1827.
- Gallagher JE, Heinrich U, George M, Hendee L, Phillips PH, Lewtas JL. 1994. Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. *Carcinogenesis* 15:1291-1299.
- Gallagher JE, Jackson M, George G, Lewtas J. 1990. Dose-related differences in DNA adduct levels in rodent tissues following skin application of complex mixtures from air pollution sources. *Carcinogenesis* 11:63-68.
- Glatt H, Henschler R, Phillips DH, Blake JW, Steinberg P, Seidel A, Oesch F. 1990. Sulphotransferase-mediated chlorination of 1-hydroxy-methylpyrene to a mutagen capable of penetrating indicator cells. *Environ Health Perspect* 88:43-48.
- Green FHY, Boyd RL, Danner-Rabovsky J, Fisher MJ, Moormen WJ, Ong T-M, Tucker J, Vallgathen V, Whong W-Z, Zoldak J, Lewtas J. 1983. Inhalation studies of diesel exhaust and coal dust in rats. *Scand J Work Environ Health* 9:181-188.
- Grimmer G, Brune H, Deutsch-Wenzel R, Dettbarn G, Jacob J, Naujack KW, Mohr U, Ernst H. 1987. Contribution of polycyclic aromatic hydrocarbons and nitro-derivatives to the carcinogenic impact of diesel engine exhaust condensate evaluated by implantation into the lungs of rats. *Cancer Lett* 37:173-180.
- Gu ZW, Zhong BZ, Nath B, Whong WZ, Wallace WE, Ong T-M. 1992. Micronucleus induction and phagocytosis in mammalian cells treated with diesel emission particles. *Mutat Res* 279:55-60.
- Guerrero RR, Rounds DE, Orthoefer J. 1981. Genotoxicity of Syrian lung cells treated in vivo with diesel exhaust particulates. *Environ Int* 5:445-454.
- Gupta RC, Reddy MV, Randerath K. 1985. Enhanced sensitivity of  $^{32}\text{P}$ -postlabelling analysis of aromatic carcinogen-DNA adducts. *Cancer Res* 45:5656-5662.
- Hasegawa NM, Nishi Y, Tsuda H, Inui N, Morimoto K. 1988. Effect of diesel exhaust particles on chromosomal aberration, sister chromatid exchanges, and morphological transformation in cultured mammalian cells. *Cancer Lett* 42:61-66.
- Hecht SS, El-Bayoumy K. 1991. The possible role of nitroarenes in human cancer. In: *Nitroarenes: Occurrence, Metabolism and Biological Impact* (Howard PC, ed.) pp. 309-316. Plenum Press, New York, NY.
- Heinrich U. 1994. Carcinogenic effects of solid particles. In: *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract*. International Life Sciences Institute (ILSI) Monographs (Mohr U, Dungworth DL, Mauderly JL, Oberdörster G, eds.) pp. 57-73. International Life Sciences Institute Press, Washington, DC.
- Hemminki K. 1993. DNA Adducts, mutations and cancer. *Carcinogenesis* 14:2007-2012.
- Huisingh J, Bradow R, Jungers R, Claxton L, Zweidinger R, Tejada S, Bumgarner J, Duffield F, Waters M, Simmon VF, Hare C, Rodrigues C, Snow L. 1978. 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* (Waters MO, Nesnow S, Huisingh JL, Sandhu SS, Claxton L, eds.) pp. 381-418. Plenum Press, New York, NY.
- International Agency for Research on Cancer. 1980. Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement No. 2, Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1983. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 32, Polynuclear Aromatic Compounds, Chemical, Environmental and Experimental Data. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1987. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vols. 1-42, Overall Evaluations of Carcinogenicity: An Update of IARC Monographs. International International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1988. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 89, Methods for Detecting DNA Damaging Agents in

- Humans: Application in Cancer Epidemiology and Prevention. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1989. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 46. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1993. Post-Labeling Methods for Detection of DNA Adducts. IARC Scientific Publication No. 124 (Phillips DH, Castegnaro M, Bartsch H, eds.). International Agency for Research on Cancer, Lyon, France.
- Iwagawa M, Maeda T, Izumi K, Otsuka H, Nishifuji K, Ohnishi Y, Aoki S. 1989. Comparative dose-response study on the pulmonary carcinogenicity of 1,6-dinitropyrene and benzo[a]pyrene in F344 rats. *Carcinogenesis* 10:1285-1290.
- Jacob J. 1990. Sulfur Analogues of Polycyclic Aromatic Hydrocarbons (Thiaarenes): Environmental Occurrence, Chemical and Biological Properties. Cambridge Monographs on Cancer Research. Cambridge University Press, Cambridge, England.
- Kawabata Y, Iwai K, Udagawa T, Tukagoshi K, Higuchi K. 1986. Effects of diesel soot on unscheduled DNA synthesis of tracheal epithelium and lung tumor formation. *Dev Toxicol Environ Sci* 13:213-222.
- Keane MJ, Xing SG, Harrison JC, Ong T, Wallace WE. 1991. Genotoxicity of diesel-exhaust particles dispersed in simulated pulmonary surfactant. *Mutat Res* 260:233-238.
- King LC, Kohan MJ, Austin AC, Claxton LD, Lewtas J, Huisinsh J. 1981. Evaluation of the release of mutagens from diesel particles in the presence of physiological fluids. *Environ Mutagen* 5:577-588.
- LaVoie EJ, Coleman DT, Geddic NG, Rice JE. 1985. Studies in the mutagenicity and tumor initiating activity of methylated fluorenes. *Chem Biol Interact* 52:301-309.
- Lee H, Cherng SH, Liu TY. 1994. Bacterial mutagenicity and DNA adduct formation by binary mixtures of benzo(a)pyrene and 1-nitropyrene. *Environ Mol Mutagen* 24:229-234.
- Levin DE, Hollstein M, Christman MF, Schwiens EA, Ames BN. 1982. A new *Salmonella* tester strain (TA 102) with A-T base pairs at the site of mutation detects oxidative mutagens. *Proc Natl Acad Sci USA* 79:7445-7449.
- Levsen K, Schilhabel J, Puttrins U. 1987. Progress in the analysis of diesel soot particles. *J Aerosol Sci* 845-848.
- Lewis TR, Green FH, Moorman WJ, Burg JA, Lynch DW. 1986. A chronic inhalation toxicity study of diesel engine emissions and coal dust, alone and combined. *Dev Toxicol Environ Sci* 13:361-380.
- Lewtas J. 1983. Evaluation of the mutagenicity and carcinogenicity of motor vehicle emissions in short-term bioassays. *Environ Health Perspect* 48:141-152.
- Lewtas J, Williams K. 1986. A retrospective view of the value of short-term genetic bioassays in predicting the chronic effects of diesel soot. *Dev Toxicol Environ Sci* 13:119-140.
- Li AP, Royer RE. 1982. Diesel exhaust particle extract enhancement of chemical induced mutagenesis in cultured Chinese hamster ovary cells: Possible interaction of diesel exhaust with environmental carcinogens. *Mutat Res* 103:349-355.
- Li D, Randerath K. 1990. Strain differences of I-compounds in relation to organ sites of spontaneous tumorigenesis and non-neoplastic renal disease in mice. *Carcinogenesis* 11:251-255.
- Liber HL, Andon BM, Hites RA, Thilly WG. 1981. Diesel soot: Mutation measurements in bacterial and human cells. *Environ Int* 5:281-284.
- MacCrehan WA, May WE, Yang SD, Brenner BA. 1988. Determination of nitro polynuclear aromatic hydrocarbons in air and diesel particulate matter using liquid chromatography with electrochemical and fluorescence detection. *Anal Chem* 60:194-199.
- Manabe YT, Kinouchi T, Ohinishi Y. 1985. Identification and quantification of highly mutagenic nitroacetoxypyrenes and nitrohydroxypyrenes in diesel exhaust particles. *Mutat Res* 158:3-15.
- Marnett LJ, Burcham PC. 1993. Endogenous DNA adducts: Potential and paradox. *Chem Res Toxicol* 6:771-785.
- Matsushita H, Goto S, Endo O, Lee J, Kawai A. 1986. Mutagenicity of diesel exhaust and related chemicals. In: *Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust* (Ishinishi N, Koizumi A, McClellan RO, Stöber W, eds.) pp. 103-118. Elsevier Science Publishing Co., New York, NY.
- Mauderly JL, Jones RK, McClellan RO, Henderson RF, Griffith WC. 1987. Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. *Fundam Appl Toxicol* 9:208-221.

- Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang IY, Cheng YS, Gillet NA, Griffith WC, Henderson RF, Mitchell CE, Nikula KJ, Thomassen DG. 1994. Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats: Part I. Neoplastic and nonneoplastic lung lesions. Research Report No. 68. Health Effects Institute, Cambridge, MA.
- McCann JE, Choi E, Yamasaki E, Ames BN. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 72:5135-5139.
- McCoy EC, Anders M, Rosenkranz HS. 1983. The basis of the insensitivity of *Salmonella typhimurium* strain TA98/1,8-DNP<sub>6</sub> to the mutagenic action of nitroarenes. Mutat Res 121:17-23.
- McCoy EC, Holloway M, Frierson G, Klopman G, Rosenkranz HS. 1985. Genetic and quantum chemical basis of the mutagenicity of nitroarenes for adenine-thymine base pairs. Mutat Res 149:311-319.
- Mermelstein R, Kiriazides DK, Butter M, McCoy EC, Rosenkranz HS. 1981. The extraordinary mutagenicity of nitropyrenes in bacteria. Mutat Res 89:187-196.
- Mitchell AD, Evans EL, Jutz MM, Riccio ES, Mustelmans KE, Simmon VF. 1981. Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions in in vitro mutagenesis and DNA damage. Environ Int 5:393-401.
- Moorthy B, Sriram P, Randerath K. 1994. Chemical structure- and time-dependent effects of polycyclic aromatic hydrocarbon-type inducers on rat liver cytochrome P450, DNA adducts, and I-compounds. Fundam Appl Toxicol 22: 549-560.
- Morimoto K, Kitamura M, Kondo H, Koizumi A. 1986. Genotoxicity of diesel exhaust emissions in a battery of in-vitro short-term bioassay. Dev Toxicol Environ Sci 13:85-101.
- Nakagawa R, Kitamori S, Horikawa K, Nakashima K, Tokiwa H. 1983. Identification of dinitropyrenes in diesel-exhaust particles: Their probable presence as the major mutagens. Mutat Res 124:201-211.
- Nesnow S, Ross H, Nelson G, Holden K, Erexson G, Kligerman A, Gupta RC. 1993. Quantitative and temporal relationship between DNA adduct formation in target and surrogate tissues: Implications for biomonitoring. Environ Health Perspect 101(3):37-42.
- Nordenson IA, Sweins A, Dahlgreen E, Beckman L. 1981. A study of chromosomal aberrations in miners exposed to diesel exhausts. Scand J Work Environ Health 7:14-17.
- Ong T-M, Whong WZ, Xu J, Burchell B, Green FHY, Lewis T. 1985. Genotoxicity studies of rodents exposed to coal dust and diesel emission particulates. Environ Res 37:399-409.
- Paputa-Peck MC, Marano RS, Schuetzle D. 1983. Determination of nitrated polynuclear aromatic hydrocarbons in particulate extracts by capillary column gas chromatography with nitrogen selective detection. Anal Chem 55:146-154.
- Pederson TC, Siak J-S. 1981. The role of nitroaromatic compounds in the direct-acting mutagenicity of diesel particle extracts. J Appl Toxicol 1:54-60.
- Pepelko WE, Peirano WB. 1983. Health effects of exposure to diesel engine emissions: A summary of animal studies conducted by the US EPA's Health Effects Research Laboratories at Cincinnati, OH. J Am Coll Toxicol 2:253-306.
- Pereira MA. 1982. Genotoxicity of diesel exhaust emissions in laboratory animals. In: Toxicological Effects of Emissions from Diesel Engines (Lewtas J, ed.) pp. 265-279. Elsevier Science Publishing Co., New York, NY.
- Piegorsch WW, Hoel DG. 1988. Exploring relationships between mutagenic and carcinogenic potencies. Mutat Res 196:161-175.
- Raat WK. 1988. Polycyclic aromatic hydrocarbons and mutagens in ambient air particles. Toxicol Environ Chem 16:259-279.
- Randerath E, Danna TF, Randerath K. 1992a. DNA damage induced by cigarette smoke condensate in vitro as assayed by <sup>32</sup>P-postlabeling: Comparison with cigarette smoke-associated DNA adduct profiles in vivo. Mutat Res 268:139-153.
- Randerath K, Li D, Moorthy B, Randerath E. 1993. I compounds—endogenous DNA markers of nutritional status, tumor promotion and carcinogenesis. In: Postlabelling Methods for Detection of DNA Adducts. International Agency for Research on Cancer Scientific Publication No. 124 (Phillips DH, Castegnaro M, Bartsch H, eds.) pp. 157-165. International Agency for Research on Cancer, Lyon, France.
- Randerath K, Li D, Nath R, Randerath K. 1992b. Exogenous and endogenous DNA modifications as monitored by <sup>32</sup>P-postlabelling: Relationships to cancer and aging. Exp Gerontol 27:533-549.

- Randerath K, Li D, Randerath E. 1990. Age-related DNA modifications (I-compounds): Modulation by physiological and pathological processes. *Mutat Res* 238:245-253.
- Randerath K, Putnam KL, Mauderly JL, Williams P, Randerath E. 1995. Pulmonary toxicity of inhaled diesel exhaust and carbon black in chronically exposed rats. Part II: Diesel exhaust and DNA damage. Research Report No. 68. Health Effects Institute, Cambridge, MA (in preparation).
- Randerath K, Reddy MV, Disher RM. 1986. Age and tissue-related DNA modifications in untreated rats: Detection by  $^{32}\text{P}$ -postlabelling assay and possible significance for spontaneous tumor induction and aging. *Carcinogenesis* 7:1615-1617.
- Randerath K, Van Golen KL, Dragan YP, Pitot HC. 1992c. Effects of phenobarbital on I-compounds in liver DNA as a function of age in male rats fed two different diets. *Carcinogenesis* 13:125-130.
- Rannug U, Sundvall A, Westerholm R, Alsberg T, Stenberg U. 1983. Some aspects of mutagenicity testing of the particulate phase and the gas phase of diluted and undiluted automobile exhaust. *Environ Sci Res* 27:3-16.
- Reddy MV, Randerath K. 1984.  $^{32}\text{P}$ -postlabelling test for covalent DNA binding of chemicals in vivo: Application to a variety of aromatic carcinogens and methylating agents. *Carcinogenesis* 5:231-243.
- Reddy MV, Randerath K. 1986. Nuclease P1-mediated enhancement of sensitivity of  $^{32}\text{P}$ -postlabelling test for structurally diverse DNA adducts. *Carcinogenesis* 7:1543-1551.
- Rice JE, Rivenson A, Braley J, LaVoie EJ. 1987. Methylated derivatives of pyrene and fluorene: Evaluation of genotoxicity in the hepatocyte/DNA repair test and tumorigenic activity in new born mice. *J Toxicol Environ Health* 21:525-532.
- Rosenkranz HS. 1993. Revisiting the role of mutagenesis in the induction of lung cancers in rats by diesel emissions. *Mutat Res* 303:91-95.
- Rosenkranz HS, Ennever FK. 1990. An association between mutagenicity and carcinogenic potency. *Mutat Res* 244:61-65.
- Rosenkranz HS, McCoy EC, Mermelstein R. 1983. Microbial assays in research and the characterization of complex mixtures. In: *Short Term Bioassays in the Analysis of Complex Environmental Mixtures, III* (Waters MD, Sandhu SS, Lewtas J, Claxton L, Chernoff N, Nesnow S, eds.) pp. 103-138. Plenum Press, New York, NY.
- Rosenkranz EJ, McCoy EC, Mermelstein R, Rosenkranz HS. 1982. Evidence for the existence of distinct nitroreductases in *Salmonella typhimurium*: Roles in mutagenesis. *Carcinogenesis* 3:121-123.
- Ross J, Nelson G, Kligerman A, Erexson G, Bryant M, Early K, Gupta RC, Nesnow S. 1990. Formation and persistence of novel benzo(a)pyrene adducts in rat lung, liver and peripheral blood lymphocyte DNA. *Cancer Res* 50:5088-5099.
- Roy AK, El-Bayoumy K, Hecht SS. 1989.  $^{32}\text{P}$ -postlabelling analysis of 1-nitropyrene-DNA adducts in female Sprague-Dawley rats. *Carcinogenesis* 10:195-198.
- Rudd CJ. 1980. Diesel particulate extract in cultured mammalian cells. In: *Health Effects of Diesel Engine Emissions* (Pepelko WE, Danner RM, Clarke NA, eds.) pp. 385-403. EPA/600/9-80/057a. U.S. Environmental Protection Agency, Cincinnati, OH.
- Saleem I, Durisin AM, Prater TJ, Riley T, Schuetzle D. 1982. Contribution of 1-nitropyrene to direct-acting Ames assay mutagenicities of diesel particulate extracts. *Mutat Res* 104:17-23.
- Saleem I, Pero AM, Zator R, Schuetzle D, Riley TL. 1984. Ames assay chromatograms and the identification of mutagens in diesel-particulate extracts. *Environ Sci Technol* 18:375-382.
- Scheepers PTJ, Bos RP. 1992a Combustion of diesel fuel from a toxicological perspective: I. Origin of incomplete combustion products. *Int Arch Occup Environ Health* 64:149-161.
- Scheepers PTJ, Bos RP. 1992b Combustion of diesel fuel from a toxicological perspective: II. Toxicology. *Int Arch Occup Environ Health* 64:162-177.
- Schenker MB, Kado NY, Hammond SK, Samuels SJ, Woskie SR, Smith TJ. 1992. Urinary mutagenic activity in workers exposed to diesel exhaust. *Environ Res* 52:133-148.
- Schuetzle D. 1983. Sampling of vehicle emissions for chemical analysis and biological testing. *Environ Health Perspect* 47:65-80.
- Schuetzle D, Jensen TE, Ball JC. 1985. Polar polynuclear aromatic hydrocarbon derivatives in extracts of particulates: Biological characterization and techniques for chemical analysis. *Environ Int* 11:169-181.
- Schuetzle D, Lee FSC, Prater TJ, Tejada SB. 1981. The identification of polynuclear aromatic hydrocarbons (PAHs) derivatives in mutagenic fractions of diesel particulate extracts. *Int J Environ Anal Chem* 9:93-144.

- Schuler RL, Niemeier RW. 1981. A study of diesel emissions on *Drosophila*. *Environ Int* 5:431-434.
- Siak J-S, Chan JL, Lee PS. 1981. Diesel particulate extracts in bacterial test systems. *Environ Int* 5:243-248.
- Smith BA, Fullerton NF, Aidoo A, Heflich RH, Beland FA. 1993. DNA adduct formation in relation to lymphocyte mutations and lung tumor induction in F344 rats treated with the environmental pollutant 1,6-dinitropyrene. *Environ Health Perspect* 99:277-280.
- Smith BA, Korfmacher WA, Beland FA. 1990. DNA adduct formation in target tissues of Sprague-Dawley rats, CD-1 mice, and A/J mice following tumorigenic doses of 1-nitropyrene. *Carcinogenesis* 11:1705-1710.
- Stump F, Bradow R, Ray W, Dropkin D, Zwedinger R, Sigsby J, Snow R. 1982. Trapping gaseous hydrocarbons for mutagenic testing. Paper No. 820776. Society of Automotive Engineers, Warrendale, PA.
- Tokiwa H, Otofujii T, Nakagawa R, Horikawa K, Maeda T, Sano N, Izumi K, Otsuka H. 1986. Dinitro derivatives of pyrene and fluoranthene in diesel emission particulates and their tumorigenicity in mice. In: *Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust* (Ishinishi N, Koizumi A, McClellan RO, Stöber W, eds.) pp. 253-270. Elsevier Science Publishing Co., Amsterdam.
- Tornquist M, Osterman-Golkar S, Kautainen A, Jensen S, Farmer PB, Ehrenberg L. 1986. Tissue dose of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. *Carcinogenesis* 7:1519-1521.
- Tucker JD, Xu J, Stewart J, Baciu PC, Ong T. 1986. Detection of sister chromatid exchanges induced by volatile genotoxins. *Teratogen Carcinogen Mutagen* 6:15-21.
- Wallace WE, Keane HJ, Hill CA, Xu J, Ong TM. 1987. Mutagenicity of diesel exhaust particles and oil shale particles dispersed with lecithin surfactant. *J Toxicol Environ Health* 21:163-171.
- Weinberg RA. 1991. Tumor suppressor genes. *Science* 254:1138-1146.
- Weyand EH, Boyla P, Wu Y, He Z, LaVoie EJ. 1993b. Detection of the major DNA adducts of benzo(*f*)fluoranthene in mouse skin: Nonclassical dihydrodiol epoxides. *Chem Res Toxicol* 6:117-124.
- Weyand EH, Cai ZW, Wu Y, Rice JH, He ZM, LaVoie EJ. 1993a. Detection of the major DNA adducts by benzo(*b*)fluoranthene in mouse skin: Role of phenolic dihydrodiol epoxides. *Chem Res Toxicol* 6:568-577.
- Whong WZ, Stewart JD, Cutler D, Ong T. 1994. Induction of in vivo DNA adducts by 4 industrial by-products in the rat lung cell system. *Mutat Res* 312:165-172.
- Willems MI, Raat WK, Wesstra JA, Bakker GL, Dubois G, van Dokkum W. 1989. Urinary and fecal mutagenicity in car mechanics exposed to diesel exhaust and in unexposed office workers. *Mutat Res* 222:375-391.
- Williams R, Sparacino C, Petersen B, Bumgarner J, Jungers RHJ, Lewtas J. 1986. Comparative characterization of organic emissions from diesel particles, coke oven mains, roofing tar vapors and cigarette smoke condensate. *Int J Environ Anal Chem* 26:27-49.
- Wolff RK, Henderson RF, Harkema JR, Mauderly JL. 1990. Pulmonary inflammation and DNA adducts in rats inhaling diesel exhaust or carbon black. *Inhal Toxicol* 2:241-254.
- Wong D, Mitchell CE, Wolff RK, Mauderly JL, Jeffrey AM. 1986. Identification of DNA damage as a result of exposure of rats to diesel engine exhaust. *Carcinogenesis* 7:1595-1597.
- Xu GB, Yu CP. 1987. Deposition of diesel exhaust particles in mammalian lungs: A comparison between rodents and man. *Aerosol Sci Technol* 7:117-123.
- Yu ML, Hites RA. 1981. Identification of organic compounds on diesel engine soot. *Anal Chem* 3:951-954.

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**ABBREVIATIONS**


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BaP	benzo[ <i>a</i> ]pyrene
CHO	Chinese hamster ovary
cpm	counts per minute
HPRT	hypoxanthine phosphoribosyl transferase
I-compounds	indigenous compounds
PAH	polycyclic aromatic hydrocarbon
RAL	relative adduct labeling
SA7	simian adenovirus
SCE	sister chromatid exchange
TiO <sub>2</sub>	titanium dioxide

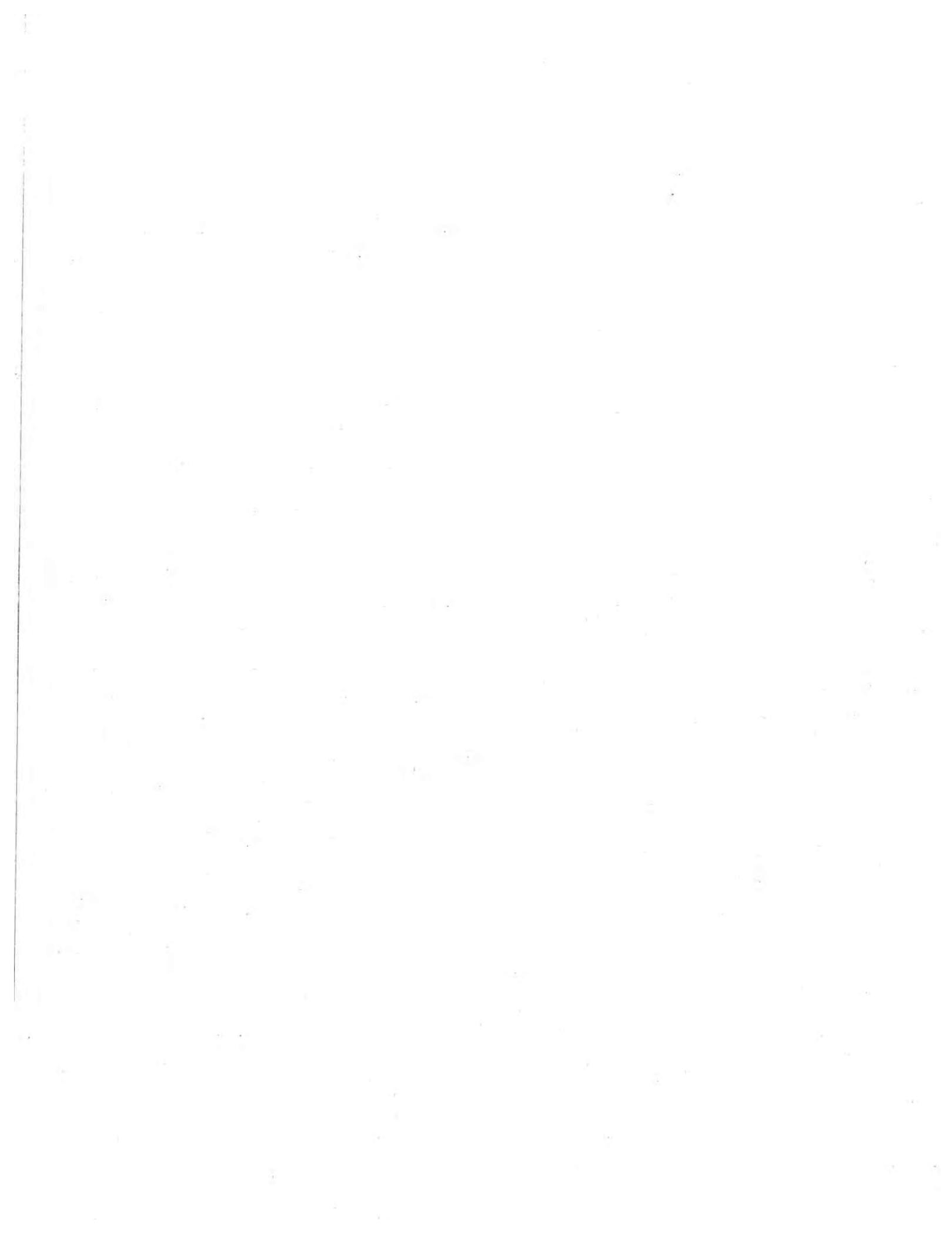
# Genotoxicity of Diesel Emissions

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## Part II: The Possible Role of Dinitropyrenes in Lung Cancer

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Diesel engine emissions contain extremely potent mutagenic species (e.g., dinitropyrene, benzo[a]pyrene), some of which also exhibit a broad spectrum of carcinogenic activities in rodents (Rosenkranz and Mermelstein 1985; Tokiwa and Ohnishi 1986; International Agency for Research on Cancer 1987, 1989). Because the mutagenic and other polycyclic organic species in diesel emissions are primarily absorbed onto or incorporated into particulate matter, the extent of their bioavailability, and therefore their role in carcinogenesis, is a matter of considerable uncertainty.

After a number of studies reported that exposure of rats to diesel engine emissions for two years resulted in the development of lung tumors (reviewed by Mauderly 1992), attention focused on the mechanistic nature of this carcinogenic process. Given that certain chemicals present in diesel engine emissions are known to be potent mutagens, the possibility existed that the induced tumors were a consequence of "genotoxic" carcinogenic events. This issue is relevant to human health risk assessment because mutagenic (genotoxic) carcinogens are thought to pose a greater health hazard than nongenotoxic ones (Williams 1987; Ashby and Morrod 1991). As a group, genotoxic carcinogens are known to cause cancer in numerous species (Ashby and Tennant 1988; Gold et al. 1989), and are more potent rodent carcinogens (Rosenkranz and Ennever 1990; Parodi et al. 1991). Moreover, the majority of recognized human carcinogens are mutagens (Ennever et al. 1987; Shelby 1988; Bartsch and Malaveille 1989). In addition,  $^{32}\text{P}$ -postlabeling experiments using DNA derived from animals exposed to diesel exhaust (Wong et al. 1986; Schoket et al. 1989; Bond et al. 1990; Carmichael et al. 1990; Gallagher et al. 1994) indicate that not only are the chemicals in diesel engine emissions bioavailable to some extent, but they can be metabolically activated to DNA-reactive species.

The problem with the genotoxic hypothesis concerns the amount of dinitropyrenes, benzo[a]pyrene, or total mutagenic substances present in diesel engine emissions as well as the emissions exposure required to induce lung cancers in rats (even assuming that all the mutagenic substances are deposited at the ultimate sensitive target tissue area and that all of the material is bioavailable—which it is not). Studies that involved calculating the amount of dinitropyrene or the total amount of mutagenic material deposited in the lungs found the level insufficient to account for the carcinogenicity of diesel engine emissions (Rosenkranz 1993; Beland 1995; see also Heinrich et al. 1992). It should be noted that these analyses did not take into consideration the differences in mode of administration (i.e., a single dose of pure chemicals versus a two-year exposure to diesel emissions).

The results of recent bioassays have shifted attention from genotoxic to nongenotoxic mechanisms of carcinogenesis. Studies have indicated that lung tumors resulting from exposure to diesel engine emissions occurred only when exposures were sufficiently high to cause reduced pulmonary clearance ("lung overload") accompanied by inflammatory and fibrotic responses (Mauderly 1992; Snipes et al. 1992; Heinrich et al. 1994; Mauderly et al. 1994). This raises the distinct possibility that the lung cancers induced in rats by exposure to diesel engine emissions were not the result of a mutagenic process (i.e., not the result of a genotoxic, DNA-adduct-forming carcinogenesis in the classic sense). This development suggests the need to examine alternative mechanisms involved in the induction of tumors by exposure to these chemical species.

The recently developed model of cell proliferation (mitogenesis) offers a possible acceptable alternative as part of a multistage model for studying carcinogenesis (Ames and Gold 1990; Butterworth 1990; Cohen and Ellwein 1990, 1991; Preston-Martin et al. 1990; Butterworth et al. 1992; International Agency for Research on Cancer 1992). Taking this model as representing the basic mechanism that determines later biochemical or cellular events, the inflammatory process that results from lung overburden can be envisioned as causing cell proliferation. It has already been demonstrated that the process of cell proliferation may generate frequent mutations. Indications are that either spontaneous mutations occur during the process of cell replication or mutations are induced by the oxidative processes that accompany the inflammatory response to lung overload (Ames and Gold 1991; Arroyo et al. 1992; Shigenaga and Ames 1993). Conceivably, some of the mutations resulting from these induced cellular responses could lead to the activation of oncogenes or the inactivation of suppressor genes. In this regard, further consideration needs to be given to the fact that diesel engine emissions contain bioavailable mutagens (see Gallagher et al. 1994), even though they cannot account quantitatively for the carcinogenicity of the diesel extract in rats.

Chemicals present in diesel engine emissions are potent mutagens (Rosenkranz and Mermelstein 1985; Tokiwa and Ohnishi 1986) that are capable of inducing oncogenes or inactivating suppressor genes (Tahira et al. 1986; Ochiai et al. 1985; Harris 1991). 1,6-Dinitropyrene, one of the most potent mutagens present in diesel emissions, has recently been shown to be an effective rat mutagen following intratracheal instillation (Smith et al. 1993; Beland 1995) under conditions demonstrated to result in 1,6-dinitropyrene-induced lung tumors (Iwagawa et al. 1989). Indeed, it was shown that such mutations were induced at levels below what is needed to induce cancer (Beland 1995). Moreover,

under the conditions of the diesel inhalation cancer bioassay, it was also calculated that there was sufficient 1,6-dinitropyrene deposited, if bioavailable, to cause somatic mutations, even though there was insufficient 1,6-dinitropyrene to account for the observed carcinogenicity (Beland 1995). Thus, although lung tumor induction in rats is not a mutagenic process, it may be one involving cell proliferation or an inflammatory response. Moreover, the possibility exists that mutagenic events caused by components in diesel engine emissions affect the progression of cancer in its later phases. These events could include oncogenic activation and suppressor inactivation, which can be amplified by cell proliferative processes (Anderson et al. 1992; Conolly 1992). The possible involvement of such augmented mutagenic events is of concern given our knowledge that in humans tumors that contain activated oncogenes or inactivated suppressor genes are generally more invasive or are associated with a poorer prognosis than histologically similar tumors lacking such activated oncogenes or inactivated suppressor genes.

Thus several analyses suggest that lung cancers induced in rats by exposure to emissions from diesel engines are not primarily the result of a mutagenic effect of the organic soluble chemicals absorbed onto the diesel particles (Rosenkranz 1993; Gallagher et al. 1994; Beland 1995). This thinking is consistent with the observation that carbon black particles, which contain greatly reduced levels of adsorbed organic mutagens, induce tumors that appear to be similar to, and develop at approximately the same levels of lung burden as those induced by diesel engine emissions (Heinrich 1994; Mauderly et al. 1994). Additionally, the latency (approximately two years) of the tumors induced by diesel engine emissions is comparable to that associated with carbon black but not to that associated with pure chemicals present in diesel emissions, such as benzo[*a*]pyrene and 1,6-dinitropyrene (approximately 0.8 year) (Iwagawa et al. 1989; see also Tokiwa et al. 1984; Takayama et al. 1985; Maeda et al. 1986; Imaida et al. 1991).

The results of recent rat bioassays (Heinrich 1994; Mauderly et al. 1994) raise a vexing question: If the dinitropyrenes present in the emissions of diesel engines are capable of causing somatic mutations (Smith et al. 1993; Beland 1995) that could initiate a carcinogenic event, why are the tumors induced in the treated animals characteristic not of dinitropyrene-induced tumors, but rather of tumors induced by particulate matter? This question raises the intriguing possibility that the conditions of the diesel emissions cancer bioassay may have precluded the ability of dinitropyrenes to induce cancers. It is known that the mutagenicity (and presumably the carcinogenicity) of dinitropyrenes requires their reduction to the corresponding arylhydroxylamines, followed by

*O*-esterification and the formation of reactive arylnitrenium species, which react at the C-8 position of the deoxyguanosine in the DNA (Smith et al. 1993; Beland 1995). Indeed, in a recent study (Beland 1995) of rats intratracheally instilled with 1,6-dinitropyrene, the C-8-DNA adduct was the only one found at the site of deposition as well as in distal tissues. This finding indicates that such bioactivation of DNA-adducting metabolites occurs under conditions relevant to exposure to diesel engine emissions when overloading is not expected to be a factor. Moreover, speculation that 1,6-dinitropyrene deposited in the lungs is more bioavailable than previously assumed is based on studies showing that radioactive 1,6-dinitropyrene migrates from the site of intratracheal deposition (Smith et al. 1993; Beland 1995). This finding was unexpected, given the relative insolubility of 1,6-dinitropyrene, and suggests that the pulmonary milieu may facilitate the diffusion of 1,6-dinitropyrene, possibly as a result of surfactant action.

Why then did the animals exposed to diesel emissions not develop tumors characteristic of 1,6-dinitropyrene exposure? Possibly the conditions of exposure to high concentrations of diesel exhaust precluded the biotransformation of 1,6-dinitropyrene to DNA-reactive species.

It is known that gases present in diesel engine emission cause "oxidative stress" (Kleinman et al. 1993), which may result in a decreased ability to reduce the nitro moiety. Moreover, oxidative stress is accompanied by a decreased level of ascorbic acid (Blaurock et al. 1992), which is needed to prevent the oxidation of the arylhydroxylamine to the corresponding nitrosoarene, a form that is incapable of reacting with DNA (Boldt et al. 1991).

In the later phase of exposure to diesel engine emissions, the inflammatory response attributable to the accumulation of particles may have the same net effect as an oxidative stress (Blaurock et al. 1992; Kleinman et al. 1993; Sagai et al. 1993) and prevent the activation of dinitropyrenes. Whereas this explanation is speculative, a number of simple experiments could be conducted to determine whether oxidative stress in rats interferes with the metabolism of dinitropyrenes to DNA-reactive species (see below).

The possibility that the conditions of the rat bioassay precluded expression of the carcinogenic potential of dinitropyrenes is, of course, directly relevant to assessing the risk to humans of exposure to diesel engine emissions when oxidative stress is not a factor. Even when occupationally exposed, humans are rarely exposed to toxic levels of gaseous emissions, and inflammatory (fibrotic) reactions due to lung "overload" have not been reported.

Epidemiological analysis of populations occupationally exposed to diesel engine emissions suggests a small, incre-

mental risk of associated lung cancer (International Agency for Research on Cancer 1989; Mauderly 1992; see also Cohen and Higgins, this report). This increased risk, however, is not expected to be associated with compromised lung functions resulting from the reduced clearance rates characteristic of rats chronically exposed to elevated levels of diesel emissions throughout their lifetime. Further, the levels of gaseous components of diesel engine emissions are not expected to be sufficient to induce oxidative stress in humans. Thus the possibility exists that human exposure to diesel engine emissions in occupational settings may involve a risk that is not accounted for by the rat model. In addition, although considerable data are available on the carcinogenic consequence in rats of chronic exposure to elevated levels of particulate matter, no evidence indicates that the rat is a good model for studying this type of lung carcinogenesis in other species, including humans.

In summary, nitrated polycyclic aromatic hydrocarbons, formed from reaction of the unsubstituted hydrocarbons with nitrogen oxides during diesel fuel combustion, are known to be metabolically activated mutagens. Metabolic transformation of nitrated polycyclic aromatic hydrocarbons to mutagenic metabolites occurs through nitroreduction to reactive *N*-hydroxyarylamines, as well as through further metabolism to their reactive *O*-acetylated derivatives, which react with C-8 of deoxyguanosine (Beland 1995).

Diesel exhaust particles have been shown to produce both superoxide anions and hydroxyl radicals *in vitro* in the absence of biological activating systems (Sagai et al. 1993). After these particles are washed with methanol, they no longer have the ability to produce reactive oxygen species, indicating that in their native state they contain organic-soluble components that are active oxidants. These agents may be important in regard to the acute toxicity of diesel particulate matter, given that particles in their native state cause considerably more mortality in mice after being instilled intratracheally than do methanol-washed particles.

Hence, although they are carriers of the potent mutagenic dinitropyrenes, diesel particles are composed also of chemicals that could induce oxidative stress in cells. Differences in the development times and types of carcinomas induced by 1,6-dinitropyrene alone and diesel particles containing 1,6-dinitropyrene suggest that the nitroaromatics may not be the causative agents for lung cancer development in rats. Possibly the active oxidants in the particles cause oxidative stress in the affected cells, conceivably contributing to carcinogenesis. If a cell is experiencing oxidative stress, the *N*-hydroxyarylamines will rapidly oxidize to their arylnitroso derivatives, which react with electrophiles on proteins (especially protein thiols) but not DNA. Indeed, if a cell is experiencing high

levels of oxidative stress, little or no cellular thiols or reductants will be available for reaction with the arylnitroso compound, and little or no protein or DNA damage by 1,6-dinitropyrene will result.

With respect to the pivotal role assigned to ascorbate in maintaining the DNA-reactivity of dinitropyrene metabolites, it should be noted that intracellular concentrations of ascorbate (usually within the submillimolar range) are about one order of magnitude lower than those of glutathione (with the notable exception of neutrophils in which ascorbate may be as high as 1.0 to 1.5 mM) (Wolf 1993). Thus ascorbate is in great excess when compared with the expected level of 1,6-dinitropyrene in tissues.

Oxidative stress produced by oxygen radicals or organic (e.g., phenoxyl) radicals is known to result in a depletion of intracellular reductants. Ascorbate is the primary reductant of these radicals. Once ascorbate is depleted, low molecular weight thiols and protein sulfhydryls become involved in the reduction of radicals (Buettner and Jurkiewicz 1993; Kagan et al. 1994). Thus, under conditions of oxidative stress, dramatically reduced levels of ascorbate may be insufficient to counteract the autooxidation of *N*-hydroxy-1-amino-6-nitropyrene to a non-DNA-reactive product.

Finally, it should be noted that damage to DNA may occur by an alternative mechanism; namely, depletion of cellular reductants, generation of reactive oxygen species, and formation of promutagenic lesions on DNA by the reactive oxygen species or their more long-lived products (Seto et al. 1994).

Future research on carcinogenicity associated with exposure to diesel exhaust should address the following questions:

1. What is the role of oxidative-stress-induced depletion of endogenous reductants (e.g., ascorbate) in modulating the mutagenic efficiency of 1,6-dinitropyrene in cells?
2. What are the roles of diesel particles and oxidants in oxidative-stress-induced promutagenic lesions?
3. What are the interactions at the cellular and molecular levels between oxidative-stress-induced DNA damage and 1,6-dinitropyrene-DNA adducts that could explain the *in vivo* tumorigenesis in rats chronically exposed to high concentrations of diesel exhaust?

The level of oxidative stress that a cell, organ, or animal is experiencing can be quantified by measurement of reductants (i.e., antioxidants) present in the system. All three questions may be answered with carefully designed short-term *in vivo* experiments, as well as *in vitro* experiments with lung cells in culture, in which the molecular products

of 1,6-dinitropyrene (e.g., DNA adducts, thiol adducts, metabolites) and oxidative stress (e.g., DNA oxidation products and adducts, levels of active antioxidants) are measured while the levels of cellular reductants, 1,6-dinitropyrene, and diesel particulate matter are controlled.

## REFERENCES

- Ames BN, Gold LS. 1991. Endogenous mutagens and the causes of aging and cancer. *Mutat Res* 250:3-16.
- Ames BN, Gold LS. 1990. Chemical carcinogenesis: Too many rodent carcinogens. *Proc Natl Acad Sci USA* 87:7772-7776.
- Anderson MW, Reynolds SH, You M, Maronpot RM. 1992. Role of proto-oncogene activation in carcinogenesis. *Environ Health Perspect* 98:13-24.
- Arroyo PL, Hatch-Pigott V, Mower HF, Cooney RV. 1992. Mutagenicity of nitric oxide and its inhibition by antioxidants. *Mutat Res* 281:193-202.
- Ashby J, Morrod RS. 1991. Detection of human carcinogens. *Nature* 352:185-186.
- Ashby J, Tennant RW. 1988. Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI/NTP. *Mutat Res* 204:17-115.
- Bartsch H, Malaveille C. 1989. Prevalence of genotoxic chemicals among animal and human carcinogens evaluated in the IARC Monograph Series. *Cell Biol Toxicol* 5:115-127.
- Beland FA. 1995. Relationship between administered dose, DNA adducts, and mutation induction in vivo. Research Report No. 72. Health Effects Institute, Cambridge, MA (in press).
- Blaurock B, Hippell S, Metz N, Elstner EF. 1992. Oxidative destruction of biomolecules by gasoline engine exhaust products and detoxifying effects of the three-way catalytic converter. *Arch Toxicol* 66:681-687.
- Boldt J, Mah MC, Wang YC, Smith BA, Beland FA, Maher VM, McCormick JJ. 1991. Kinds of mutations found when a shuttle vector containing adducts of 1,6-dinitropyrene replicates in human cells. *Carcinogenesis* 12(1):119-26.
- Bond JA, Johnson NF, Snipes MB, Mauderly JL. 1990. DNA adduct formation in rat alveolar type II cells: Cells potentially at risk for inhaled diesel exhaust. *Environ Mol Mutagen* 16:64-69.
- Buettner GR, Jurkiewicz BA. 1993. Ascorbate free radical as a marker of oxidative stress: An EPR study. *Free Radic Biol Med* 14:49-55.
- Butterworth BE. 1990. Consideration of both genotoxic and nongenotoxic mechanisms in predicting carcinogenic potential. *Mutat Res* 239:117-132.
- Butterworth BE, Popp JA, Conolly RB, Goldsworthy TL. 1992. Chemically induced cell proliferation in carcinogenesis. In: *Mechanisms of Carcinogenesis in Risk Identification* (Vainio H, Magee P, McGregor D, McMichael AJ, eds.) pp. 279-305. IARC Scientific Publication No. 116. International Agency for Research on Cancer, Lyon, France.
- Carmichael PL, Jacob J, Grimmer G, Phillips DH. 1990. Analysis of the polycyclic aromatic hydrocarbon content of petrol and diesel engine lubricating oils and determination of DNA adducts in topically treated mice by <sup>32</sup>P-postlabelling. *Carcinogenesis* 11:2025-2032.
- Cohen SM, Ellwein LB. 1990. Cell proliferation in carcinogenesis. *Science* 249:1007-1011.
- Cohen SM, Ellwein LB. 1991. Genetic errors, cell proliferation and carcinogenesis. *Cancer Res* 51:6493-6505.
- Conolly RB. 1992. A biologically-based risk assessment strategy for inhaled formaldehyde. *Comments Toxicol* 4(4):269-293.
- Ennever FK, Noonan TJ, Rosenkranz, HS. 1987. The predictivity of animal bioassays and short-term genotoxicity tests for carcinogenicity and non-carcinogenicity to humans. *Mutagenesis* 2:73-78.
- Gallagher J, Heinrich U, George M, Hendee L, Phillips DH, Lewtas J. 1994. Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. *Carcinogenesis* 15:1291-1299.
- Gold LS, Bernstein L, Magaw R, Slone TH. 1989. Interspecies extrapolation in carcinogenesis: Prediction between rats and mice. *Environ Health Perspect* 81:211-219.
- Harris CC. 1991. Chemical and physical carcinogenesis: Advances and perspectives for the 1990s. *Cancer Res* 51:5023s-5044s.
- Heinrich U. 1994. Effects of solid particles. In: *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract* (Dungworth DL, Mauderly JL, Obendorfster G, eds.) International Life Sciences Institute (ILSI) Press, Washington, DC.

- Heinrich U, Fuhst R, Dasenbrock C, Muhle H, Koch W, Mohr U. Long-term inhalation exposure of rats and mice to diesel exhaust, carbon black, and titanium dioxide (abstract). The Ninth Health Effects Institute Annual Conference, p. 15. Health Effects Institute, Cambridge, MA.
- Imaida K, Lee MS, Wang CY, King CM. 1991. Carcinogenicity of dinitropyrenes in the weaning female CD rat. *Carcinogenesis* 12:1187-1191.
- International Agency for Research on Cancer. 1987. Monographs on the Evaluation of Carcinogenic Risks to Humans, Suppl. 6, Genetic and related effects. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1989. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 46, Diesel and gasoline engine exhausts and some nitroarenes. International Agency for Research on Cancer, Lyon, France.
- International Agency for Research on Cancer. 1992. Mechanisms of Carcinogenesis in Risk Identification (Vainio H, Magee P, McGregor D, McMichael AJ, eds.). IARC Scientific Publication No. 116. International Agency for Research on Cancer, Lyon, France.
- Iwagawa M, Maeda T, Izumi K, Otsuka H, Nishifuji K, Ohnishi Y, Aoki S. 1989. Comparative dose-response study on the pulmonary carcinogenicity of 1,6-dinitropyrene and benzo(a)pyrene in F344 rats. *Carcinogenesis* 10:1285-1290.
- Kagan VE, Yalowich JC, Day BW, Goldman R, Stoyanovsky DA. 1994. Ascorbate is the primary reductant of the phenoxyl radical of etoposide (VP-16) in the presence of thiols both in cell homogenates and in model systems. *Biochemistry* 33:9651-9660.
- Kleinman MT, Bhalla DK, Ziegler B, Bucherevans S, McClure T. 1993. Effects of inhaled fine particles and ozone on pulmonary macrophages and epithelia. *Inhalation Toxicol* 5:371-388.
- Maeda T, Izumi K, Otsuka H, Manabe Y, Kinouchi T, Ohnishi Y. 1986. Induction of squamous cell carcinoma in the rat lung by 1,6-dinitropyrene. *J Natl Cancer Inst* 76:693-701.
- Mauderly JL. 1992. Diesel exhaust. In: *Environmental Toxicants: Human Exposures and Their Health Effects* (Lippmann M, ed.) pp. 119-162. Van Nostrand Reinhold, New York, NY.
- Mauderly JL, Snipes MB, Barr EB, Belinsky SA, Bond JA, Brooks AL, Chang IY, Cheng YS, Gillett NA, Griffith WC, Henderson RF, Mitchell CE, Nikula KJ, Thomassen DG. Pulmonary Toxicity of Inhaled Diesel Exhaust and Carbon Black in Chronically Exposed Rats, Part I: Neoplastic and Nonneoplastic Lung Lesions. 1994. Research Report No. 68. Health Effects Institute, Cambridge, MA.
- Ochiai M, Nagao M, Tahira, T, Ishikawa F, Hayashi K, Ohgaki H, Terada M, Tsuchida N, Suginura T. 1985. Activation of K-ras and oncogenes other than ras family in rat fibrosarcomas induced by 1,8-dinitropyrene. *Cancer Lett* 29:119-125.
- Parodi S, Malacarne D, Romano P, Taningher M. 1991. Are genotoxic carcinogens more potent than nongenotoxic carcinogens? *Environ Health Perspect* 95:199-204.
- Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE. 1990. Increased cell division as a cause of human cancer. *Cancer Res* 50:7415-7421.
- Rosenkranz HS. 1993. Revisiting the role of mutagenesis in the induction of lung cancers in rats by diesel emissions. *Mutat Res* 303:91-95.
- Rosenkranz HS, Ennever FK. 1990. An association between mutagenicity and carcinogenic potency. *Mutat Res* 244:61-65.
- Rosenkranz HS, Mermelstein R. 1985. The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons. *Environ Carcinogen Rev, J Environ Sci Health, Pt. C3*:221-272.
- Sagai M, Saito H, Ichinose T, Kodama M, Mori Y. 1993. Biological effects of diesel exhaust particles: I. In vitro production of superoxide and in vivo toxicity in mouse. *Free Radic Biol Med* 14:37-47.
- Schocket B, Hewer A, Grover PL, Phillips DH. 1989. <sup>32</sup>P-post-labelling analysis of DNA adducts in the skin of mice treated with petrol and diesel engine lubricating oils and exhaust condensates. *Carcinogenesis* 10:1485-1490.
- Seto H, Ohkubo T, Koike H, Saito M, Sasano H. 1994. Significant formation of 8-hydroxydeoxyguanosine through interaction of diesel particulate matter with deoxyguanosine. *Bull Environ Contam Toxicol* 53:789-795.
- Shelby MD. 1988. The genetic toxicity of human carcinogens and its implications. *Mutat Res* 204:3-15.
- Shigenaga MK, Ames BN. 1993. Oxidants and mitogenesis as causes of mutation and cancer: the influence of diet. *Basic Life Sci* 61:419-436.

- Smith BA, Fullerton NF, Aidoo A, Heflich RH, Beland FA. 1993. DNA adduct formation in relation to lymphocyte mutations and lung tumor induction in F344 rats treated with the environmental pollutant, 1,6-dinitropyrene. *Environ Health Perspect* 99:277-280.
- Snipes MB, Barr, EB, Chang I-Y, Henderson RF, Nikula KJ, Mauderly JL. 1992. Particle clearance and sequestration in lungs of rats exposed chronically to diesel exhaust or carbon black. In: Abstracts, The Ninth Health Effects Institute Annual Conference, Monterey, CA, p. 20.
- Tahira T, Hayashi K, Ochiai M, Tsuchida N, Nagao M, Suginura T. 1986. Structure of the c-Ki-ras gene in rat fibrosarcoma induced by 1,8-dinitropyrene. *Mol Cell Biol* 6:1349-1351.
- Takayama S, Ishikawa T, Nakajima H, Sato S. 1985. Lung carcinoma induction in Syrian golden hamsters by intratracheal instillation of 1,6-dinitropyrene. *Jpn J Cancer Res (Gann)* 76:457-461.
- Tokiwa H, Ohnishi Y. 1986. Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *Crit Rev Toxicol* 17:23-69.
- Tokiwa H, Otofujii T, Horikawa K, Kitamori S, Otsuka H, Manabe Y, Kinouchi T, Ohnishi Y. 1984. 1,6-Dinitropyrene: Mutagenicity in *Salmonella* and carcinogenicity in BALB/c mice. *J Natl Cancer Inst* 73:1359-1363.
- Williams GM. 1987. Definition of a human cancer hazard. In: *Nongenotoxic Mechanisms in Carcinogenesis*, Banbury Report 25 (Butterworth BE, Slaga TJ, eds.) pp. 367-380. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Wolf G. 1993. Uptake of ascorbic acid by human neutrophils. *Nutr Rev* 51(11):33708.
- Wong D, Mitchell CE, Wolff RK, Mauderly JL, Jeffrey AM. 1986. Identification of DNA damage as a result of exposure of rats to diesel engine exhaust. *Carcinogenesis* 7:1595-1597.

# Health Effects of Diesel Exhaust: Epidemiology

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Introduction	253
Lung Cancer	253
Exposure	253
Cigarette Smoking	256
Cohort and Case-Control Studies of Occupational Groups	256
General Population Studies	266
Discussion	269
Conclusion	269
Other Cancers	270
Occupational Cohorts	270
General Population Studies	271
Discussion	274
Nonmalignant Respiratory Disease	276
Studies of Respiratory Morbidity	276
Mortality Studies	281
Discussion	283
Paper Summary	284
Acknowledgments	284
References	285
Endnotes	289
Abbreviations	292



## INTRODUCTION

This background paper assesses the epidemiologic evidence concerning the health effects of exposure to whole diesel exhaust. Because whole diesel exhaust contains carcinogens (see background papers by Busby and Newberne, Rosenkranz, Shirnamé-Moré, and Sawyer and Johnson, this report), epidemiologists have focused their attention on cancer in exposed populations, especially cancers of the lung and bladder. A smaller body of epidemiologic research addresses effects on lung function and respiratory symptoms. In virtually all studies, only occupational exposure is considered.

This paper is divided into five main sections. Epidemiologic data are reviewed in three sections covering lung cancer, other cancers, and nonmalignant respiratory disease. A summary at the end of the paper presents conclusions. References are listed in the final section of the chapter, followed by a list of abbreviations.

In compiling this paper, we reviewed all published epidemiologic studies on the health effects of exposure to diesel exhaust available through June 1993. We identified these studies by searching MEDLINE (the National Library of Medicine's online bibliographic data base) and by reviewing the reference sections of published research and earlier reviews. The major results for all studies are presented in at least tabular form, and a number of the most recent cohort or case-control studies of occupations that involve exposure to diesel exhaust are discussed in detail.

## LUNG CANCER

Epidemiologists began to study the relation between lung cancer and occupational exposure to diesel exhaust in the 1950s (Table 1), when the U.S. railroad and trucking industries and public mass transit widely converted to diesel-powered engines. Several reviews discuss these studies, including Steenland (1986), who covers studies published through 1985; the International Agency for Research on Cancer (1989), which covers most studies published through 1988; and Mauderly (1992), who covers studies published through 1990. All reviewers note the modest relative increases in lung cancer incidence or mortality observed in association with exposure to diesel exhaust, but acknowledge a paucity of data on exposure and potential confounding due to cigarette smoking that, as described below, make some results difficult to interpret.

## EXPOSURE

No study has ascertained levels of exposure to diesel exhaust for the actual study subjects, not an uncommon

situation for many epidemiologic studies of putative occupational carcinogens. In most cases, historical estimates of exposure levels in specific job categories, which can be used by epidemiologists to estimate past exposures, are not available, and no standard method to measure exposure is yet available (see Watts paper, this report). This state of affairs makes it difficult to use the results of epidemiologic studies for quantitative risk assessment, but does not at all preclude their usefulness for more qualitative inference.

Most investigators have classified exposure based on subject-reported work histories or on company or other records. Typically, those jobs and occupations considered by the investigator to entail exposure to diesel exhaust are classified as exposed. Whereas this approach is probably sensitive to diesel exhaust exposure (i.e., it tends to identify most truly exposed subjects), it is not specific (i.e., it tends to classify some truly unexposed subjects as exposed). This misclassification of exposure may produce biased results in an individual study and may suggest that results from different studies are not in agreement (Rothman 1986). Such misclassification can spuriously elevate or diminish estimates of effects, depending on how misclassification differs between subjects with and without disease. In most cases, when the extent of misclassification of exposure is the same for those with and without disease (i.e., nondifferential misclassification), estimates of effect are attenuated. When the risk of disease increases directly and monotonically with exposure (sometimes referred to as a "dose response"), nondifferential misclassification of exposure can obscure this pattern (Dosemeci et al. 1990; Birkett 1992).

Epidemiologists have studied populations of workers in industries or occupations where diesel-powered vehicles are frequently used, or have studied general populations and attempted to identify individuals within them with occupational exposure to diesel exhaust. These design options have different implications with respect to the assessment of exposure to diesel exhaust. The quality of information on exposure to diesel exhaust is generally superior in studies of worker populations because, to characterize exposure, they can often draw upon work histories and sometimes estimates of exposure associated with various jobs. In addition, the prevalence of exposure, particularly intense or prolonged exposure, is greater. General population studies, however, often include large numbers of people and provide detailed information on cigarette smoking from direct interviews. To improve the specificity of exposure characterization and to increase the statistical power of general population studies, investigators often aggregate various occupations when calculating estimates

**Table 1.** Epidemiologic Studies of Lung Cancer and Diesel Exhaust

Reference	Population	Study Type	Cases or Deaths	Exposure Assessment	Smoking Controlled	Relative Risk (95% CI)
Raffle 1957	London (England) transport workers	Cohort mortality	30	Job records	No	1.42 (0.96, 2.02)
Kaplan 1959	Railroad workers	Cohort mortality	154 Total 49 Most exposed	Job records	No	0.80 (0.68, 0.94) Total cohort 0.88 (0.65, 1.16) Most exposed
Waxweiler et al. 1973	Potash miners	Cohort mortality	31	Diesel use in mines	No	None reported
Menck and Henderson 1976	Los Angeles county (U.S.)	Cohort mortality	109	Death certificates: hospital records	No	1.65 (1.36, 1.99) Truckers
Decoufle et al. 1977	Hospital-based	Case-control mortality	66	Occupation by questionnaire	Yes	0.92 (NA) <sup>a</sup> Truck, bus, taxi drivers 0.97 (NA) Railroad workers
Williams et al. 1977	U.S. Third National Cancer Survey	Case-control incidence	22	Occupation by interviews	Yes	1.52 (NA) Truckers
Leupker and Smith 1978	U.S. Teamsters Union	Cohort mortality	34	Union records	No	1.21 (0.84, 1.69)
Ahlberg et al. 1981	Swedish truckers	Cohort mortality	1,143	Job records	No	1.3 (1.1, 1.6) Truckers
Waller 1981	London (England) transport workers	Cohort mortality	667 Total 177 Engineers	Job records	No	0.79 (0.73, 0.85) Total cohort 0.90 (0.77, 1.04) Bus garage engineers
Howe et al. 1983	Canadian railroad workers	Cohort mortality	933 Total 279 Probably exposed	Job held at retirement	No	1.06 (0.99, 1.13) Total 1.35 (NA) Probably exposed
Milne et al. 1983	Alameda County, CA (U.S.)	Case-control mortality	NA	Occupation from death certificates	No	1.6 (NA) Truckers
Rushlon et al. 1983	London (England) bus garage workers	Cohort mortality	102 Total 48 General hand	Job records	No	1.01 (0.82, 1.22) Total 1.33 (0.98, 1.76) General hand
Coggon et al. 1984	English	Case-control mortality	598 Total exposed 172 High exposure	Death certificates	No	1.3 (NA) Total 1.1 High exposure
Hall and Wynder 1984	U.S. hospital-based	Case-control incidence	45 Exposed	Usual occupation by interviews	Yes	1.4 (0.8, 2.4)
Schenker et al. 1984	U.S. railroad workers	Cohort mortality	2,519 Total	Job records	No	1.4 (0.5, 2.4)
Buiatti et al. 1985	Italian hospital-based	Case-control incidence	20 Ever employed as taxi driver	Occupation by interviews	Yes	1.8 (1.0, 3.4)
Damber and Larson 1985, 1987	Swedish	Case-control mortality	33 Professional drivers 20+ yr	Occupational history by mailed questionnaire	Yes	1.1 (0.6, 2.2)
Wong et al. 1985	U.S. heavy-equipment operators	Cohort mortality	309 Total 163 20+ yr in union	Job title from union records	No	0.98 (0.88, 1.10) 1.07 (0.92, 1.25) 20+ Years
Gustafsson et al. 1986	Swedish dockworkers	Cohort incidence and mortality	70 Deaths 86 Cases	Job records	No	1.32 (1.05, 1.66) Deaths 1.68 (1.36, 2.07) Cases
Edling et al. 1987	Swedish bus company employees	Cohort mortality	6 Total 5 Bus drivers 1 Bus garage worker 0 Clerks (unexposed)	Job records	No	0.67 (0.27, 1.39) Total cohort
Garshick et al. 1987	U.S. railroad workers	Case-control mortality	335 Cases ≤ 64 yr 480 Cases ≥ 65 yr	Job records and particle level measurements	Yes	1.41 (1.06, 1.88) ≤ 64 yr/20+ yr 0.91 (NA) ≥ 65 yr/20+ yr

(Table continues next page.)

**Table 1.** Epidemiologic Studies of Lung Cancer and Diesel Exhaust (*continued*)

Reference	Population	Study Type	Cases or Deaths	Exposure Assessment	Smoking Controlled	Relative Risk (95% CI)
Lerchen et al. 1987	New Mexico (U.S.)	Case-control incidence	5 Mechanics 7 Total exposed	Occupation by interviews	Yes	0.6 (0.2, 2.0) Mechanics 0.6 (0.2, 1.6) Total exposed
Benhamou et al. 1988	French	Case-control incidence	65 Mechanics 157 Transport equipment operators 128 Professional drivers	Occupation by questionnaire	Yes	1.06 (0.73, 1.54) Mechanics 1.35 (1.05, 1.75) Operators 1.42 (1.07, 1.89) Drivers
Boffetta et al. 1988	American Cancer Society cohort—U.S. males	Cohort mortality	174 Exposed 14 Railroad workers 48 Truckers	Occupational exposure by questionnaire	Yes	1.18 (0.97, 1.44) Exposed 1.59 (0.94, 2.69) Railroad workers 1.24 (0.93, 1.66) Truckers
Garshick et al. 1988	U.S. railroad workers	Cohort mortality	1,694 Total	Job records and particle level measurements	No	1.45 (1.11, 1.89) (40-44 yr in 1959) 0.99 (0.74, 1.33) (60-64 yr in 1959) 1.72 (1.27, 2.33) (15+ yr exposure)
Siemiatycki et al. 1988	Montreal (Canada) hospital-based	Case-control incidence	81 Squamous cell	Occupational exposure by interviews and job/exposure matrix	Yes	1.2 (0.9, 1.6)
Bender et al. 1989	Minnesota (U.S.) highway maintenance workers	Cohort mortality	54	Employment in highway maintenance department	No	0.69 (0.52, 0.90)
Hayes et al. 1989	U.S. hospital-based	Case-control incidence	112 Truckers 10+ yr 18 Mechanics 10+ yr	Occupation by interviews	Yes	1.5 (1.1, 2.0) Truckers 2.1 (0.9, 5.2) Mechanics
Boffetta et al. 1990	U.S. hospital-based	Case-control incidence	477 Self-reported exposure 12 Self-reported 31+ yr	Occupation by interviews	Yes	1.21 (0.73, 2.02) Exposed 2.39 (0.87, 6.57) 31+ yr
Gustavsson et al. 1990	Swedish bus garage workers	Cohort and nested case-control incidence and mortality studies	17 Deaths 20 Cases	Semiquantitative scale from job records and industrial hygiene data	No	Cohort: SMR = 1.22 (0.71, 1.96) Case-control: 2.43 (1.32, 4.47) Highest cumulative exposure
Steenland et al. 1990	U.S. Central States Teamsters Union	Case-control mortality	730 Truckers 50 Mechanics	Union records, next-of-kin interviews, and particle level measurements	Yes	1.27 (0.83, 1.93) Long-haul 1.31 (0.81, 2.11) Short-haul 1.69 (0.92, 3.09) Mechanics
Ahlman et al. 1991	Finnish sulfide ore miners	Cohort mortality	10	Occupational histories	No	1.45 (0.74, 2.58)
Burns and Swanson 1991	Detroit metropolitan (U.S.)	Case-control incidence	118 Mechanics 35 Railroad workers 166 Drivers of heavy-duty trucks	Occupational history from interviews with subject or proxy	Yes	1.72 (1.15, 2.59) Mechanics 1.37 (0.70, 2.66) Railroad workers 2.31 (1.56, 3.42) Drivers
Emmelin et al. 1993	Swedish dockworkers	Case-control incidence	50	Semiquantitative scales from work histories and records of fuel and equipment use	Yes	(1.0) Low exposure <sup>b</sup> 2.7 (0.6, 11.3) Medium exposure 6.8 (1.3, 34.9) High exposure

<sup>a</sup> NA = not available.<sup>b</sup> Relative risk estimates and 90% CIs are taken from Table 3 (exposure metric based on exposed time) in reference.

of effects. This can obscure risk elevations if occupations with minimal exposure are combined with high-exposure occupations. Most general population studies also provide estimates of the relative risks for specific occupations, however, which can be compared to the estimates from studies of occupational cohorts. In this review, we emphasize studies of worker populations and give particular emphasis to several recent studies that attempted to improve exposure estimates by using work histories, personal interviews, and information on historical and contemporary working conditions (including, in some cases, levels of constituents of diesel exhaust). We then review general population studies and discuss their findings in the light of the studies of worker populations.

### CIGARETTE SMOKING

Cigarette smoking plays a role in the occurrence of most lung cancer in the United States and other western countries, and its effect is clearly independent of other environmental exposures and occupational factors. Studies of the effect of diesel exhaust exposure on lung cancer occurrence must account for the strong independent effect of cigarette smoking, because the effect of diesel exhaust on lung cancer may be either exaggerated or understated if exposed and unexposed populations have different smoking habits. This mixing of the effects of two or more independent causes of disease, known as confounding, can be controlled analytically if data are available on the smoking habits of study subjects (Rothman 1986). In epidemiologic studies of occupational carcinogens, such data frequently are not available, and several of the studies discussed below did not ascertain the smoking habits of their participants. Nonetheless, such studies may still provide useful information if the magnitude of confounding by cigarette smoking can be estimated.

We have used methods described by Axelson (1978) to quantify the likely effect of uncontrolled confounding by cigarette smoking. Briefly, these methods call for using ancillary data to provide estimates of the prevalence of smoking in the study population and the expected relative risk of lung cancer due to smoking in the absence of diesel exhaust exposure. These data are then used to compute an estimated relative risk due to confounding that can be compared to the observed relative risk. If the observed relative risk exceeds the estimated relative risk due to confounding, the evidence suggests that confounding by cigarette smoking cannot account for the observed increase in risk.

It is also clear that cigarette smoking can act synergistically with other factors, such as asbestos (Hughes and Weill

1994), to increase the incidence of lung cancer to a greater extent than would be expected by simply adding the effects of each. Such interactions between smoking and other causes of lung cancer are potentially important because so many people have been or currently are smokers. Such interactions are difficult to study epidemiologically, however, because they require estimates of the risk of lung cancer among unexposed subjects who are also nonsmokers, and there are often few people who have never smoked in the groups most often exposed to diesel exhaust.

### COHORT AND CASE-CONTROL STUDIES OF OCCUPATIONAL GROUPS

Epidemiologists have used several approaches to study lung cancer in diesel exhaust-exposed populations. They have conducted studies of entire working populations, or cohort studies, in which the rates of lung cancer mortality or morbidity are compared between exposed and unexposed subjects. Often the unexposed subjects are the general population of the country or another political division, but in some cases comparisons of risk are made between exposed and unexposed (or less exposed) members of the cohort. Such internal comparisons are often preferred because they are less subject to distortion due to differences in health status between employed and general populations, known as the healthy worker effect. The result of either comparison is usually expressed as a rate ratio or relative risk, and the statistical precision of the relative risk is expressed as a confidence interval.

The case-control approach provides investigators with an efficient way to estimate the relative risk of lung cancer in relation to diesel exhaust exposure without having to collect information on entire populations. In the case-control study, cases of lung cancer that occurred in the study population are ascertained and classified according to diesel exhaust exposure; then a sample of the study population (the control subjects) is selected and classified as to exposure. An estimate of the relative risk then can be calculated from these data. More detailed discussions of these study designs can be found in texts by Rothman (1986) and Checkoway and colleagues (1989).

### Railroad Retirement Board Studies

Garshick and colleagues studied lung cancer mortality among U.S. railroad workers registered with the Railroad Retirement Board (RRB)\* in both cohort (Garshick et al. 1988) and case-control (Garshick et al. 1987) studies.

In conjunction with these investigations, Woskie and colleagues (1988a,b) conducted an industrial hygiene sur-

\* A list of abbreviations appears at the end of this background paper.

vey under current conditions to characterize the exposure of railroad workers to diesel exhaust in four small northern U.S. railroad operations. Industrial hygienists first identified 39 job titles (from among the more than 150 Interstate Commerce Commission [ICC] railroad job titles) that comprised large numbers of workers and that were thought to involve either minimal or substantial exposure to diesel exhaust. The investigators then measured contemporary levels of respirable particles [RP] and adjusted them for cigarette smoke (adjusted respirable particles [ARP], a surrogate measurement for the particle phase of diesel exhaust) for each job title; they then grouped the 39 job titles into five career exposure categories based on similarities in work environment and patterns of career advancement (Woskie 1988a,b) (Table 2). The absolute ARP levels, although adjusted for cigarette smoke particles, also reflect the presence of non-diesel exhaust particles (Woskie 1988a,b; see Watts background paper, this report). Nonetheless, they probably provide an accurate relative ranking of diesel exhaust exposure (Woskie 1988a,b).

In both the cohort and the case-control studies, Garshick and colleagues divided workers into two exposure groups: an exposed group consisting of shop workers, engineers, firemen, brakemen, conductors, and hostlers, and an unexposed group consisting of clerical workers and signalmen. The Woskie and colleagues studies generally corroborate this dichotomous ranking, but also suggest that members of each group did not all experience the same exposure (Table 2) (Woskie et al. 1988a,b; Watts paper, this report). For example, the jobs classified by Garshick and colleagues as exposed show twofold differences in mean levels of ARP, and signalmen have higher levels of particle exposure than clerks, though both were classified by Garshick and colleagues as unexposed. Nevertheless, any measurement error or misclassification, if nondifferential between lung cancer decedents and control subjects, would result only in an under-estimate of a truly elevated relative risk.

**Table 2.** Diesel Exhaust Exposure Classification for the Railroad Retirement Board Cohort<sup>a</sup>

Career Exposure Group	Average Adjusted Respirable Particles Level ( $\mu\text{g}/\text{m}^3$ and 95% CI)	Classification in Epidemiologic Studies
Clerks	33 (31, 35)	Unexposed
Signal maintainers	58 (50, 64)	Unexposed
Engineers/firemen	71 (65, 77)	Exposed
Brakemen/conductors	89 (83, 95)	Exposed
Shopworkers	141 (125, 157)	Exposed

<sup>a</sup> Data are from Woskie et al. (1988b), Table II.

**Railroad Retirement Board Cohort Study: Garshick and Colleagues (1988).** The introduction of diesel engines in the U.S. railroad industry began on a nationwide basis in approximately 1949. Garshick and colleagues ascertained the mortality through 1980 of 55,407 white male railroad workers who were 40 to 64 years of age in 1959 (Table 3) when nationwide conversion to diesel railway engines was virtually complete. Subjects were required to have begun work between 10 and 20 years earlier (i.e., between 1939 and 1949) and to have been employed in 1959 in one of the 39 jobs surveyed in the industrial hygiene study (Woskie 1988a,b). Workers who retired close to or before 1959 had less-extended exposure to diesel exhaust than the younger workers. Railroad Retirement Board records listed each subject's job title, or titles, for each year since 1959. To reduce the potential for confounding due to asbestos exposure, Garshick and colleagues excluded from the cohort all workers whose jobs involved known exposure to asbestos (chiefly in the repair of railroad cars and the overhaul of steam-engine boilers).

Mortality was ascertained from the RRB through December 31, 1980; of 19,396 deaths, 1,694 death certificates indicated lung cancer (International Classification of Diseases [ICD] 8th Revision code 162 [World Health Organization 1965]) as a primary or contributing cause of death.

The investigators measured the effect of diesel exhaust exposure according to either the job held in 1959 or cumulative years in an exposed job (Table 4). Workers generally held one job for an entire career. The relative risks for lung cancer and diesel exhaust exposure based on the job held in 1959 are inversely related to age in 1959; workers who were 40 to 44 in 1959 and who held exposed jobs in 1959 (and hence had both the longest potential exposure to diesel exhaust and, potentially, the longest induction times between exposure and death) experienced a 46% increase (relative risk [RR] = 1.46, 95% confidence interval [CI] 1.12, 1.90) in lung cancer mortality relative to members of their birth cohort who held unexposed jobs in 1959. Excess lung cancer mortality declined with increasing age: workers who were 55 to 59 in 1959 experienced an 18% increase (RR = 1.18, 95% CI 0.93, 1.49); those 60 to 64 years old experienced no increase (RR = 0.99, 95% CI 0.74, 1.33).

Relative risks appeared to increase with years of exposure (Table 4). Workers with greater than 15 years of employment in an exposed job experienced a 72% increase in lung cancer mortality (RR = 1.72, 95% CI 1.27, 2.33). When job categories with potential asbestos exposure were excluded (i.e., shopworkers and hostlers), the relative risk for cumulative exposure of more than 15 years rose from 1.72 to 1.82 (95% CI 1.30, 2.55), perhaps because asbestos exposure might be expected to be inversely correlated with

**Table 3.** Characteristics of the Railroad Retirement Board Cohort<sup>a</sup>

Characteristic	Age in 1959				
	40-44	45-49	50-54	55-59	60-64
Cohort members	20,081	13,946	9,848	7,237	4,295
Retirement year (median)	1977	1974	1971	1967	1963
Percent exposed in 1959	75.3	77.5	73.6	72.4	69.2
Percentage of years exposed (1959-1980):					
20+	30.8	6.0	0.2	0.0	0.0
10-19	31.6	55.3	51.7	26.7	4.2
1-9	13.6	17.0	22.3	46.3	65.3
0	23.9	21.7	25.7	26.9	30.5
Percent potentially exposed to asbestos	19.7	21.9	26.4	28.1	28.5

<sup>a</sup> Data are from Garshick et al. (1988), Tables 3 and 4.

diesel exposure, having occurred primarily as a consequence of steam-engine boiler repair work in earlier years. The results of analyses of cumulative exposure, however, appear to be sensitive to modeling assumptions.<sup>1</sup>

Cigarette smoking information was not available for cohort members.<sup>2</sup> It is possible that the inverse relation between risk and birth cohort reflects not the effect of diesel exhaust exposure, but rather the effect of increasing consumption of cigarettes by younger birth cohorts.<sup>3</sup> Cigarette smoking alone, however, is unlikely to explain the excess lung cancer mortality in the youngest, and most exposed, birth cohorts (see endnote 4).

**Railroad Retirement Board Case-Control Study: Garshick and Colleagues (1987).** Garshick and colleagues con-

ducted a case-control study of RRB registrants who died between March 1, 1981, and February 28, 1982. These deaths occurred among 650,000 active and retired male railroad workers, born in or after 1900, with at least 10 years of railroad employment. Cases consisted of all deaths for which primary lung cancer (ICD 8 code 162) was indicated on the death certificate. Each case was matched to two control subjects in age within 2.5 years and date of death within 31 days. Persons who died from cancer, suicide, accidents, or unknown causes were excluded as control subjects, although deaths from nonmalignant respiratory disease were included (15% of the control subjects among older workers and 7% of control subjects among younger workers). If diesel exhaust exposure causes death from nonmalignant respiratory disease (see below), then includ-

**Table 4.** Lung Cancer Relative Risks and 95% Confidence Intervals for the Railroad Retirement Board Cohort<sup>a</sup>

Relative Risk <sup>b</sup>	Age in 1959				
	40-44	45-49	50-54	55-59	60-64
Exposure in 1959	1.46 (1.12, 1.90)	1.33 (1.03, 1.73)	1.12 (0.88, 1.42)	1.18 (0.93, 1.49)	0.99 (0.74, 1.33)
	Cumulative Years of Exposure				
	0	1-4	5-9	10-14	15+
Exposure	(1.0)	1.20 (1.01, 1.44)	1.24 (1.06, 1.44)	1.32 (1.13, 1.56)	1.72 (1.27, 2.33)
Exposure with asbestos exposure excluded	(1.0)	1.34 (1.08, 1.65)	1.33 (1.12, 1.58)	1.33 (1.10, 1.60)	1.82 (1.30, 2.55)

<sup>a</sup> Data are from Garshick et al. (1988), Figure 1 and Table 6.

<sup>b</sup> Exposure classified dichotomously, that is, exposed or unexposed.

ing these deaths among control subjects would result in an underestimation of the relative risk. However, this effect is most likely negligible in the study by Garshick and colleagues (1987).<sup>4</sup>

Garshick and colleagues assessed diesel exhaust exposure using (1) job histories beginning in 1959 for subjects who retired after 1959, and (2) the last job worked before retirement for workers who retired between 1955 and 1959. Each job was classified as exposed or unexposed, and cumulative exposure to diesel exhaust was summarized for each worker as diesel-years of exposure. Unlike the cohort study, the case-control study included workers who had worked in jobs other than any of the 39 jobs characterized for contemporary diesel exhaust levels in the industrial hygiene survey by Woskie and colleagues (1988a,b). This introduces a possible source of exposure misclassification.<sup>5</sup>

Next of kin (mostly spouses) provided information on cigarette smoking history. Information on whether the subject had ever smoked was obtained for 86% of cases and 82% of control subjects. Information on age when smoking began and pack-years of smoking history was obtained for 70% to 80% of study subjects. Asbestos exposure was assessed from the work history for those who retired after 1959 and from the last job held for those who retired between 1955 and 1959.

The investigators conducted separate analyses for workers who died at 64 years of age or younger and those at or older than 65 years of age, primarily because heavy cumulative exposure was concentrated among the younger workers (Table 3). In addition, the investigators suspected that information on the cause of death was less accurate for older workers. Among younger workers, 20 years of diesel exhaust exposure was associated with a 39% increase (RR = 1.39, 95% CI 1.05, 1.83) in lung cancer mortality before adjusting for asbestos exposure and cigarette smoking (Table 5). Adjusting for these factors hardly changed the estimated effect of 20 years of exposure (RR = 1.41, 95% CI 1.06, 1.88) (Table 5)<sup>6</sup>. A stratified analysis found a relative risk of 1.02 (95% CI 0.72, 1.45) among those with 5 to 19 years of exposure, and a relative risk of 1.64 (95% CI 1.18, 2.29) among those with 20 or more diesel-years of exposure.<sup>7</sup> No excess mortality from lung cancer in association with diesel exhaust exposure was observed among the older workers.

It is difficult to draw firm conclusions about the magnitude of the relative increase in lung cancer among the railroad workers exposed to diesel exhaust in the RRB studies because of two potential sources of error in exposure characterization. First, as noted by Garshick and colleagues (1988), the data were not analyzed according to the time elapsed since exposure began or since a particular

amount of exposure was sustained (usually referred to as latency or induction time). Because decades may be required for the effect of exposure to manifest in disease or death, the largest effects might be expected to occur in the longest induction time categories. In addition, other aspects of the timing and duration of exposure may play a role in pathogenesis. Second, the use of a dichotomous exposure classification ignores apparent differences in average ARP levels among groups of railroad workers who were considered to be uniformly exposed (see Table 2). If historical exposure levels were directly associated with risk, and if misclassification of exposure does not depend on disease status, then analyses that consider risks by exposure level might well reveal larger relative risks in association with exposure level when exposure levels are combined. The study of Schenker and colleagues (1984) may provide insight on this issue.

**Pilot Study: Schenker and Colleagues (1984).** A cohort study of 2,519 railroad workers, conducted as a pilot investigation for the RRB studies, indicates the possible effect of further refining the exposure measurements. The study included workers with the same jobs as those in the full RRB cohort. Schenker and colleagues divided the career groups, however, into categories of unexposed, and low, high, and undefined exposure, which generally correspond to the rank ordering of ARP levels measured by the industrial hygienists. In analyses that used the job held in 1959 to characterize the participant's entire career, Schenker and colleagues observed lung cancer mortality relative risks of 1.5 (95% CI 0.96, 2.35) and 2.77 (95% CI 0.91, 8.42) for low and high exposure, respectively. The ratio of incidence rates (standardized to the age and calendar period distribution of the cohort) for exposed (high plus low exposure) versus unexposed was 1.42 (95% CI 0.45, 2.39), which is consistent with the results of the full studies.

Although this pilot study suggests that further refinement of exposure measurement might be informative, Garshick has noted (Garshick 1991, personal communication) that the highly exposed shopworker group may have been substantially misclassified because not all shops repaired diesel engines.

**Reanalysis of Railroad Retirement Board Cohort Study: Crump and Colleagues (1991)<sup>8</sup>.** Crump and colleagues reanalyzed the data from the 1988 cohort study by Garshick and associates with the dual objectives of replicating the original results and conducting new analyses using more detailed information on exposure, including quantitative estimates of diesel exhaust exposure derived from the industrial hygiene measurements by Woskie and colleagues (1988a,b).