

## 4. MUTAGENICITY

1           Since 1978, more than 100 publications have appeared in which genotoxicity assays were  
2 used with diesel emissions, the volatile and particulate fractions (including extracts), or individual  
3 chemicals found in diesel emissions. Although most of the studies deal with the question of  
4 whether gas-phase or particulate extracts from diesel emissions possess mutagenic activity in  
5 microbial and mammalian cell assays, a number of studies have employed bioassays (most  
6 commonly *Salmonella* TA98 without S9) to evaluate (1) extraction procedures, (2) fuel  
7 modifications, (3) bioavailability of chemicals from diesel particulate matter (DPM), and (4)  
8 exhaust filters or other modifications and variables associated with diesel emissions. As indicated  
9 in Chapter 2, the number of chemicals in diesel emissions is very large. Many of these have been  
10 determined to exhibit mutagenic activity in a variety of assay systems (see Table II in Claxton,  
11 1983). Although a detailed discussion of those data is beyond the scope of this document, some  
12 of the mutagenically active compounds found in the gas phase are ethylene, benzene, 1,3-  
13 butadiene, acrolein, and several polycyclic aromatic hydrocarbons (PAHs) (see Table 2-21). Of  
14 the particle-associated chemicals, several PAHs and nitro-PAHs have been the focus of mutagenic  
15 investigations both in bacteria and in mammalian cell systems (see Table 2-22). Several review  
16 articles, some containing more detailed descriptions of the available studies, are available (IARC,  
17 1989; Claxton, 1983; Pepelko and Peirano, 1983; Shirnamé-Moré, 1995). Discussions of  
18 genotoxicity are also found in the proceedings of several symposia on the health effects of diesel  
19 emissions (U.S. EPA, 1980; Lewtas, 1982; Ishinishi et al., 1986).

### 4.1. GENE MUTATIONS

22           Huisinigh et al. (1978) demonstrated that dichloromethane extracts from DPM were  
23 mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and  
24 without rat liver S9 activation. This report contained data from several fractions as well as DPM  
25 from different vehicles and fuels. Similar results with diesel extracts from various engines and  
26 fuels have been reported by a number of investigators using the *Salmonella* frameshift-sensitive  
27 strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981;  
28 Brooks et al., 1984). Similarly, mutagenic activity was observed in *Salmonella* forward mutation  
29 assays measuring 8-azaguanine resistance (Claxton and Kohan, 1981) and in *E. coli* mutation  
30 assays (Lewtas, 1983).

31           One approach to identifying significant mutagens in chemically complex environmental  
32 samples such as diesel exhaust or ambient particulate extracts is the combination of short-term  
33 bioassays with chemical fractionation (Scheutzle and Lewtas, 1986). The analysis is most  
34 frequently carried out by sequential extraction with increasingly polar or binary solvents.

1 Fractionation by silica-column chromatography separates compounds by polarity or into acidic,  
2 basic, and neutral fractions. The resulting fractions are too complex to characterize by chemical  
3 methods, but the bioassay analysis can be used to determine fractions for further analysis. In most  
4 applications of this concept, *Salmonella* strain TA98 without the addition of S9 has been used as  
5 the indicator for mutagenic activity. Generally, a variety of nitrated polynuclear aromatic  
6 compounds have been found that account for a substantial portion of the mutagenicity (Liberti et  
7 al., 1984; Schuetzle and Frazer, 1986; Schuetzle and Perez, 1983). However, not all bacterial  
8 mutagenicity has been identified in this way, and the identity of the remainder of the mutagenic  
9 compounds remains unknown. The nitrated aromatics thus far identified in diesel exhaust were  
10 the subject of review in the IARC monograph on diesel exhaust (IARC, 1989).

11 In addition to the simple qualitative identification of mutagenic chemicals, several  
12 investigators have used numerical data to express mutagenic activity as activity per distance  
13 driven or mass of fuel consumed. These types of calculations have been the basis for estimates  
14 that the nitroarenes (both mono- and dinitropyrenes) contribute a significant amount of the total  
15 mutagenic activity of the whole extract (Nishioka et al., 1982; Salmeen et al., 1982; Nakagawa et  
16 al., 1983). In a 1983 review, Claxton discussed a number of factors that affected the mutagenic  
17 response in *Salmonella* assays. Citing the data from the Huisinigh et al. (1978) study, the author  
18 noted that the mutagenic response could vary by a factor of 100 using different fuels in a single  
19 diesel engine. More recently, Crebelli et al. (1995) used *Salmonella* to examine the effects of  
20 different fuel components. They reported that although mutagenicity was highly dependent on  
21 aromatic content, especially di- or triaromatics, there was no clear effect of sulfur content of the  
22 fuel. Later, Sjögren et al. (1996), using multivariate statistical methods with ten diesel fuels,  
23 concluded that the most influential chemical factors in *Salmonella* mutagenicity were sulfur  
24 contents, certain PAHs (1-nitropyrene), and naphthenes.

25 Matsushita et al. (1986) tested particle-free diesel exhaust gas and a number of benzene  
26 nitro-derivatives and PAHs (many of which have been identified as components of diesel exhaust  
27 gas). The particle-free exhaust gas was positive in both TA100 and TA98, but only without S9  
28 activation. Of the 94 nitrobenzene derivatives tested, 61 were mutagenic, and the majority  
29 showed greatest activity in TA100 without S9. Twenty-eight of 50 PAHs tested were mutagenic,  
30 all required the addition of S9 for detection, and most appeared to show a stronger response in  
31 TA100. When 1,6-dinitropyrene was mixed with various PAHs or an extract of heavy-duty (HD)  
32 diesel exhaust, the mutagenic activity in TA98 was greatly reduced when S9 was absent but was  
33 increased significantly when S9 was present. These latter results suggest that caution should be  
34 used in estimating mutagenicity (or other toxic effects) of complex mixtures from the specific  
35 activity of individual components.

1 Mitchell et al. (1981) reported mutagenic activity of DPM extracts of diesel emissions in  
2 the mouse lymphoma L5178Y mutation assay. Positive results were seen both with and without  
3 S9 activation in extracts from several different vehicles, with mutagenic activity only slightly  
4 lower in the presence of S9. These findings have been confirmed in a number of other mammalian  
5 cell systems using several different genetic markers. Casto et al. (1981), Chescheir et al. (1981),  
6 Li and Royer (1982), and Brooks et al. (1984) all reported positive responses at the HPRT locus  
7 in Chinese hamster ovary (CHO) cells. Morimoto et al. (1986) used the APRT and Oua<sup>r</sup> loci in  
8 CHO cells; Curren et al. (1981) used Oua<sup>r</sup> in BALB/c 3T3 cells. In all of these studies, mutagenic  
9 activity was observed without S9 activation. Liber et al. (1981) used the thymidine kinase (TK)  
10 locus in the TK6 human lymphoblast cell line and observed induced mutagenesis only in the  
11 presence of rat liver S9 when testing a methylene chloride extract of diesel exhaust. Barfknecht et  
12 al. (1982) also used the TK6 assay to identify some of the chemicals responsible for this  
13 activation-dependent mutagenicity. They suggested that fluoranthene, 1-methylphenanthrene, and  
14 9-methylphenanthrene could account for more than 40% of the observed activity.

15 Morimoto et al. (1986) injected DPM extracts (250 to 4,000 mg/kg) into pregnant Syrian  
16 hamsters and measured mutations at the APRT locus in embryo cells cultivated 11 days after i.p.  
17 injection. Neutral fractions from both light-duty (LD) and HD tar samples resulted in increased  
18 mutation frequency at 2,000 and 4,000 mg/kg. Belisario et al. (1984) applied the Ames test to  
19 urine from Sprague-Dawley rats exposed to single applications of DPM administered by gastric  
20 intubation, i.p. injection, or s.c. gelatin capsules. In all cases, dose-related increases were seen in  
21 TA98 (without and with S9) from urine concentrates taken 24 h after particle administration.  
22 Urine from Swiss mice exposed by inhalation to filtered exhaust (particle concentration 6 to 7  
23 mg/m<sup>3</sup>) for 7 weeks (Pereira et al., 1981a), or Fischer 344 rats exposed to DPM (2 mg/m<sup>3</sup>) for 3  
24 months to 2 years was negative in *Salmonella* strains.

25 Schuler and Niemeier (1981) exposed *Drosophila* males in a stainless steel chamber  
26 connected to the 3-m<sup>3</sup> chamber used for the chronic animal studies at EPA (see Hinnners et al.,  
27 1980, for details). Flies were exposed for 8 h and mated to untreated females 2 days later.  
28 Although the frequency of sex-linked recessive lethals from treated males was not different from  
29 that of controls, the limited sample size precluded detecting less than a threefold increase over  
30 controls. The authors noted that, because there were no signs of toxicity, the flies might tolerate  
31 exposures to higher concentrations for longer time periods.

32 Driscoll et al. (1996) exposed Fischer 344 male rats to aerosols of carbon black (1.1, 7.1  
33 and 52.8 mg/m<sup>3</sup>) or air for 13 weeks (6 h/day, 5 days/week) and measured *hprt* mutations in  
34 alveolar type II cells in animals immediately after exposure and at 12 and 32 weeks after the end  
35 of exposure. Both the two higher concentrations resulted in significant increases in mutant  
36 frequency. Whereas the mutant frequency from the 7.1 mg/m<sup>3</sup> group returned to control levels by

1 12 weeks, the mutant frequency of the high-exposure group was still higher than controls even  
2 after 32 weeks. Carbon black particles have very little adsorbed PAHs, hence a direct chemically  
3 induced mechanism is highly unlikely. Induction of *hprt* mutations were also observed in rat  
4 alveolar epithelial cells after intratracheal instillation with carbon black,  $\alpha$ -quartz and titanium  
5 dioxide (Driscoll et al., 1997). All three types of particles elicited an inflammatory response, as  
6 shown by significant increases of neutrophils in bronchalveolar lavage (BAL) fluid. Culturing the  
7 BAL from exposed rats with a rat lung epithelial cell line also resulted in elevation of *hprt*  
8 mutational response. This response was effectively eliminated when catalase was included in the  
9 incubation mixture, providing evidence for cell-derived oxidative damage.

10 Specific-locus mutations were not induced in (C3H  $\times$  101)F<sub>1</sub> male mice exposed to diesel  
11 exhaust 8 h/day, 7 days/week for either 5 or 10 weeks (Russell et al., 1980). The exhaust was a  
12 1:18 dilution and the average particle concentration was 6 mg/m<sup>3</sup>. After exposure, males were  
13 mated to T-stock females and matings continued for the reproductive life of the males. The  
14 results were unequivocally negative; no mutants were detected in 10,635 progeny derived from  
15 postspermatogonial cells or in 27,917 progeny derived from spermatogonial cells.

16 Hou et al. (1995) measured DNA adducts and *hprt* mutations in 47 bus maintenance  
17 workers and 22 control individuals. All were nonsmoking men from garages in the Stockholm  
18 area and the exposed group consisted of 16 garage workers, 25 mechanics, and 6 others. There  
19 were no exposure data but the three groups were considered to be of higher to lower exposure to  
20 diesel engine exhaust. Levels of DNA adducts determined by <sup>32</sup>P-postlabeling were significantly  
21 higher in workers than controls (3.2 versus  $2.3 \times 10^{-8}$ ), but *hprt* mutant frequencies were not  
22 different ( $8.6$  versus  $8.4 \times 10^{-6}$ ). Both adduct level and mutagenicity were highest among the 16  
23 most exposed; mutant frequency was significantly correlated with adduct level. All individuals  
24 were genotyped for glutathione transferase GSTM1 and aromatic amino transferase NAT2  
25 polymorphism. Neither GSTM1 nulls nor NAT2 slow acetylators exhibited effects on either DNA  
26 adducts or *hprt* mutant frequencies.

## 27 28 **4.2. CHROMOSOME EFFECTS**

29 Mitchell et al. (1981) and Brooks et al. (1984) reported increases in sister chromatid  
30 exchanges (SCE) in CHO cells exposed to DPM extracts of emissions from both LD and HD  
31 diesel engines. Morimoto et al. (1986) observed increased SCE from both LD and HD DPM  
32 extracts in PAH-stimulated human lymphocyte cultures. Tucker et al. (1986) exposed human  
33 peripheral lymphocyte cultures from four donors to direct diesel exhaust for up to 3 h. Exhaust  
34 was cooled by pumping through a plastic tube about 20 feet long; airflow was 1.5 L/min.  
35 Samples were taken at 16, 48, and 160 min of exposure. Cell cycle delay was observed in all  
36 cultures; significantly increased SCE levels were reported for two of the four cultures. Structural

1 chromosome aberrations were induced in CHO cells by DPM extracts from a Nissan diesel engine  
2 (Lewtas, 1983) but not by similar extracts from an Oldsmobile diesel engine (Brooks et al., 1984).

3 DPM dispersed in an aqueous mixture containing dipalmitoyl lecithin (DPL), a component  
4 of pulmonary surfactant or extracted with dichloromethane (DCM), induced similar responses in  
5 SCE assays in Chinese hamster V79 cells (Keane et al., 1991), micronucleus tests in V79 and  
6 CHO cells (Gu et al., 1992) and unscheduled DNA synthesis (UDS) in V79 cells (Gu et al.,  
7 1994). After separating the samples into supernatant and sediment fractions, mutagenic activity  
8 was confined to the sediment fraction of the DPL sample and the supernatant of the DCM sample.  
9 These findings suggest that the mutagenic activity of DPM inhaled into the lungs could be made  
10 bioavailable through solubilization and dispersion nature of pulmonary surfactants. In a later study  
11 in the same laboratory, Liu et al. (1996) found increased micronuclei in V79 cells treated with  
12 crystalline quartz and a noncrystalline silica, but response was reduced after pretreatment of the  
13 particles with the simulated pulmonary surfactant.

14 Pereira et al. (1981a) exposed female Swiss mice to diesel exhaust 8 h/day, 5 days/week  
15 for 1, 3, and 7 weeks. The incidence of micronuclei and structural aberrations was similar in bone  
16 marrow cells of both control and exposed mice. Increased incidences of micronuclei, but not  
17 SCE, were observed in bone marrow cells of male Chinese hamsters after 6 months of exposure  
18 to diesel exhaust (Pereira et al., 1981b).

19 Guerrero et al. (1981) observed a linear concentration-related increase in SCE in lung cells  
20 cultured after intratracheal instillation of DPM at doses up to 20 mg/hamster. However, they did  
21 not observe any increase in SCE after 3 months of inhalation exposure to diesel exhaust particles  
22 ( $6 \text{ mg/m}^3$ ).

23 Pereira et al. (1982) measured SCE in embryonic liver cells of Syrian hamsters. Pregnant  
24 females were exposed to diesel exhaust (containing about  $12 \text{ mg/m}^3$  particles) from days 5 to 13  
25 of gestation or injected intraperitoneally with diesel particles or particle extracts on gestational  
26 day 13 (18 h before sacrifice). Neither the incidence of SCE nor mitotic index was affected by  
27 exposure to diesel exhaust. The injection of DPM extracts but not DPM resulted in a dose-  
28 related increase in SCE; however, the toxicity of the DPM was about twofold greater than the  
29 DPM extract.

30 In the only studies with mammalian germ cells, Russell et al. (1980) reported no increase  
31 in either dominant lethals or heritable translocations in males of T-stock mice exposed by  
32 inhalation to diesel emissions. In the dominant lethal test, T-stock males were exposed for 7.5  
33 weeks and immediately mated to females of different genetic backgrounds (T-stock; [C3H  $\times$  101];  
34 [C3H  $\times$  C57BL/6]; [SEC  $\times$  C57BL/6]). There were no differences from controls in any of the  
35 parameters measured in this assay. For heritable translocation analysis, T-stock males were  
36 exposed for 4.5 weeks and mated to (SEC  $\times$  C57BL/6) females, and the  $F_1$  males were tested for

1 the presence of heritable translocations. Although no translocations were detected among 358  
2 progeny tested, the historical control incidence is less than 1/1,000.

### 3 4 **4.3. OTHER GENOTOXIC EFFECTS**

5         Pereira et al. (1981b) exposed male strain A mice to diesel exhaust emissions for 31 or 39  
6 weeks using the same exposure regimen noted in the previous section. Analyses of caudal sperm  
7 for sperm-head abnormalities were conducted independently in three separate laboratories.  
8 Although the incidence of sperm abnormalities was not significantly above controls in any of the  
9 three laboratories, there were extremely large differences in scoring among the three (control  
10 values were 9.2%, 14.9%, and 27.8% in the three laboratories). Conversely, male Chinese  
11 hamsters exposed for 6 months (Pereira et al., 1981c) exhibited almost a threefold increase in  
12 sperm-head abnormalities. It is noted that the control incidence in the Chinese hamsters was less  
13 than 0.5%. Hence, it is not clear whether the differing responses reflect true species differences  
14 or experimental artifacts.

15         A number of studies measuring DNA adducts in animals exposed to DPM, carbon black,  
16 or other particles have been reported and are reviewed by Shirnamé-Moré (1995). Although  
17 modest increases in DNA adducts have been observed in lung tissue of rats after inhalation of  
18 DPM (Wong et al., 1986; Bond et al., 1990), the increases are small in comparison with those  
19 induced by chemical carcinogens present in diesel exhaust (Smith et al., 1993). While Gallagher  
20 et al. (1994) found no increases in total DNA adducts in lung tissue of rats exposed to diesel  
21 exhaust, carbon black or titanium dioxide, they did observe an increase in an adduct with  
22 migration properties similar to nitrochrysene and nitro-benzo(a)pyrene adducts from diesel but not  
23 carbon black or titanium dioxide exposures. The majority of the studies used the <sup>32</sup>P-postlabeling  
24 assay to detect adducts. Although this method is sensitive, chemical identity of adducts can only  
25 be inferred if an adduct spot migrates to the same location as a known prepared adduct.

26         DNA adducts have also been measured in humans occupationally exposed to diesel  
27 exhaust. Distinct adduct patterns were found among garage workers occupationally exposed to  
28 diesel exhaust when compared with nonexposed controls (Nielsen and Autrup, 1994).  
29 Furthermore, the findings were concordant with the adduct patterns observed in groups exposed  
30 to low concentrations of PAHs from combustion processes. Hemminki et al. (1994) also reported  
31 significantly elevated levels of DNA adducts in lymphocytes from garage workers with known  
32 diesel exhaust exposure compared with unexposed mechanics. Hou et al. (1995) found elevated  
33 adduct levels in bus maintenance workers exposed to diesel exhaust. Although no difference in  
34 mutant frequency was observed between the groups, the adduct levels were significantly different  
35 (3.2 vs.  $2.3 \times 10^{-8}$ ). Nielsen et al. (1996) reported significantly increased levels of three

1 biomarkers (lymphocyte DNA adducts, hydroxyethylvaline adducts in hemoglobin, and 1-  
2 hydroxypyrene in urine) in DE-exposed bus garage workers.

3 The role of oxidative damage in causing mutations has received increasing focus recently.  
4 More than 50 different chemicals have been studied in rodents, usually measuring the formation of  
5 8-hydroxydeoxyguanosine (8-OH-dG), a highly mutagenic adduct (Loft et al., 1998). Increases in  
6 the mutagenic DNA adduct 8-hydroxydeoxyguanosine were found in mouse lung DNA after  
7 intratracheal instillation of diesel particles (Nagashima et al., 1995). The response was dose  
8 dependent. Mice fed on a high-fat diet showed an increased response whereas the responses were  
9 partially reduced when the antioxidant  $\beta$ -carotene was included in the diet (Ichinose et al., 1997).  
10 Oxidative damage has also been measured in rat lung tissue after intratracheal instillation of quartz  
11 (Nehls et al., 1997) and in rat alveolar macrophages after in vitro treatment with silica dust  
12 (Zhang et al., 2000). Arimoto et al. (1999) demonstrated that redissolved methanol extracts of  
13 DPM also induced the formation of 8-OH-dG adducts in L120 mouse cells. The response was  
14 dependent on both DPM concentration and P450 reductase. A detailed discussion of the potential  
15 role of oxidative damage in diesel exhaust carcinogenesis is presented in Chapter 7.4.

#### 16 17 **4.4. SUMMARY**

18 Extensive studies with *Salmonella* have unequivocally demonstrated mutagenic activity in  
19 both particulate and gaseous fractions of diesel exhaust. In most of the studies using *Salmonella*,  
20 DPM extracts and individual nitropyrenes exhibited the strongest responses in strain TA98 when  
21 no exogenous activation was provided. Gaseous fractions reportedly showed greater response in  
22 TA100, whereas benzo(a)pyrene and other unsubstituted PAHs are mutagenic only in the  
23 presence of S9 fractions. The induction of gene mutations has been reported in several in vitro  
24 mammalian cell lines after exposure to extracts of DPM. Note that only the TK6 human cell line  
25 did not give a positive response to DPM extracts in the absence of S9 activation. Mutagenic  
26 activity was recovered in urine from animals treated with DPM by gastric intubation and i.p. and  
27 s.c. implants, but not by inhalation of DPM or diluted diesel exhaust. Dilutions of whole diesel  
28 exhaust did not induce sex-linked recessive lethals in *Drosophila* or specific-locus mutations in  
29 male mouse germ cells.

30 Structural chromosome aberrations and SCE in mammalian cells have been induced by  
31 particles and extracts. Whole exhaust induced micronuclei but not SCE or structural aberrations  
32 in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 months. In a  
33 shorter exposure (7 weeks), neither micronuclei nor structural aberrations were increased in bone  
34 marrow of female Swiss mice. Likewise, whole diesel exhaust did not induce dominant lethals or  
35 heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

1 The application of mutagenicity data to the question of the potential carcinogenicity of  
2 diesel emissions is based on the premise that genetic alterations are found in all cancers and that  
3 several of the chemicals found in diesel emissions possess mutagenic activity in a variety of  
4 genetic assays. These genetic alterations can be produce by gene mutations, deletions,  
5 translocations, aneuploidy, or amplification of genes, hence no single genotoxicity assay should be  
6 expected to either qualitatively or quantitatively predict rodent carcinogenicity. With diesel  
7 emissions or other mixtures, additional complications arise because of the complexity of the  
8 material being tested. Exercises that combined the *Salmonella* mutagenic potency with the total  
9 concentration of mutagenic chemicals deposited in the lungs could not account for the observed  
10 tumor incidence in exposed rats (Rosenkranz, 1993; Goldstein et al., 1998). However, such  
11 calculations ignored the contribution of gaseous-phase chemicals which have been estimated to  
12 contribute from less than 50% (Rannug et al., 1983) to over 90% (Matsushita et al., 1986) of the  
13 total mutagenicity. This wide range is partly reflective of the differences in material tested:  
14 semivolatile extracts in the former and whole gaseous emission in the latter. Of greater  
15 importance is that these calculations are based on a reverse mutation assay in bacteria with  
16 metabolic processes strikingly different from mammals. This is at least partly reflected in the  
17 observations that different nitro-PAHs give different responses in bacteria and in CHO cells (Li  
18 and Dutcher, 1983) or in human hepatoma-derived cells (Eddy et al., 1986).

#### 20 **4.5. REFERENCES**

- 21  
22 Arimoto, T; Yoshikawa, T; Takano, H; et al. (1999) Generation of reactive oxygen species and 8-hydroxy-2'-  
23 deoxyguanosine formation from diesel exhaust particle components in L1210 cells. *Jpn J Pharmacol* 80:49-54.  
24  
25 Barfknecht, TR; Hites, RA; Cavaliers, EL; et al. (1982) Human cell mutagenicity of polycyclic aromatic  
26 hydrocarbon components of diesel emissions. In: *Toxicological effects of emissions from diesel engines:*  
27 *proceedings of the Environmental Protection Agency 1981 diesel emissions symposium; October 1981; Raleigh,*  
28 *NC. (Developments on toxicology and environmental science: v. 10.) Lewtas, J, ed. New York: Elsevier*  
29 *Biomedical; pp. 277-294.*  
30  
31 Belisario, MA; Buonocore, V; De Marinis, E; et al. (1984) Biological availability of mutagenic compounds  
32 adsorbed onto diesel exhaust particulate. *Mutat Res* 135:1-9.  
33  
34 Bond, JA; Mauderly, JL; Wolff, RK. (1990) Concentration- and time-dependent formation of DNA adducts in  
35 lungs of rats exposed to diesel exhaust. *Toxicology* 60:127-135.  
36  
37 Brooks, A; Li, AP; Dutcher, JS; et al. (1984) A comparison of genotoxicity of automobile exhaust particles from  
38 laboratory and environmental sources. *Environ Mutagen* 6:651-668.  
39  
40 Casto, BC; Hatch, GG; Huang, SL; et al. (1981) Mutagenic and carcinogenic potency of extracts of diesel and  
41 related environmental emissions: in vitro mutagenesis and oncogenic transformation. *Environ Int* 5:403-409.  
42  
43 Chescheir, GM, III; Garrett, NE; Shelburne, JD; et al. (1981) Mutagenic effects of environmental particulates in  
44 the CHO/HGPRT system. In: *Application of short-term bioassays in the fractionation and analysis of complex*

1 environmental mixtures. Waters, MD; Sandhu, SS; Huisingsh, JL; et al., eds. New York: Plenum Press; pp. 337-  
2 350.

3  
4 Claxton, LD. (1981) Mutagenic and carcinogenic potency of diesel and related environmental emissions:  
5 *Salmonella* bioassay. *Environ Int* 5:389-391.

6  
7 Claxton, LD. (1983) Characterization of automotive emissions by bacterial mutagenesis bioassay: a review.  
8 *Environ Mutagen* 5:609-631.

9  
10 Claxton, L; Kohan, M. (1981) Bacterial mutagenesis and the evaluation of mobile-source emissions. In: Short-term  
11 bioassays in the analysis of complex environmental mixtures II: proceedings of the second symposium on the  
12 application of short-term bioassays in the fractionation and analysis of complex environmental mixtures; March  
13 1980; Williamsburg, VA. (Hollaender, A; Welch, BL; Probststein, RF, eds. Environmental science research series:  
14 v. 22.) Waters, MD; Sandhu, SS; Huisingsh, JL; et al., eds. New York: Plenum Press; pp. 299-317.

15  
16 Crebelli, R; Conti, L; Crochi, B; et al. (1995) The effect of fuel composition on the mutagenicity of diesel engine  
17 exhaust. *Mutat Res* 346:167-172.

18  
19 Curren, RD; Kouri, RE; Kim, DM; et al. (1981) Mutagenic and carcinogenic potency of extracts from diesel  
20 related environmental emissions: simultaneous morphological transformation and mutagenesis in BALB/c 3T3  
21 cells. *Environ Int* 5:411-415.

22  
23 Driscoll, KE; Carter, JM; Howard, BW; et al. (1996) Pulmonary inflammatory, chemokine, and mutagenic  
24 responses in rats after subchronic inhalation of carbon black. *Toxicol Appl Pharmacol* 136:372-380.

25  
26 Driscoll, KE; Deyo, KC; Carter, JM; et al. (1997) Effects of particle exposure and particle-elicited inflammatory  
27 cells on mutation in rat alveolar epithelial cells. *Carcinogenesis* 18:423-430.

28  
29 Dukovich, M; Yasbin, RE; Lestz, SS; et al. (1981) The mutagenic and SOS-inducing potential of the soluble  
30 organic fraction collected from diesel particulate emissions. *Environ Mutagen* 3:253-264.

31  
32 Eddy, EP; McCoy, EC; Rosenkranz, HS; et al. (1986) Dichotomy in the mutagenicity and genotoxicity of  
33 nitropyrenes: apparent effect of the number of electrons involved in nitroreduction. *Mutat Res* 161:109-111.

34  
35 Gallagher, J; Heinrich, U; George, M; et al. (1994) Formation of DNA adducts in rat lung following chronic  
36 inhalation of diesel emissions, carbon black and titanium dioxide particles. *Carcinogenesis* 15:1291-1299.

37  
38 Goldstein, LS; Weyand, EH; Safe, S; et al. (1998) Tumors and DNA adducts in mice exposed to benzo[a]pyrene  
39 and coal tars: implications for risk assessment. *Environ Health Perspect* 106 Suppl 6:1325-1330.

40  
41 Gu, ZW; Zhong, BZ; Nath, B; et al. (1992) Micronucleus induction and phagocytosis in mammalian cells treated  
42 with diesel emission particles. *Mutat Res* 279:55-60.

43  
44 Gu, ZW; Zhong, BZ; Keane, MJ; et al. (1994) Induction of unscheduled DNA synthesis in V70 cells by diesel  
45 emission particles dispersed in simulated pulmonary surfactant. *Ann Occup Hyg* 38:345-349.

46  
47 Guerrero, RR; Rounds, DE; Orthofer, J. (1981) Sister chromatid exchange analysis of Syrian hamster lung cells  
48 treated in vivo with diesel exhaust particulates. *Environ Int* 5:445-454.

49  
50 Hemminki, K; Söderling, J; Ericson, P; et al. (1994) DNA adducts among personnel servicing and loading diesel  
51 vehicles. *Carcinogenesis* 15:767-769.

52  
53 Hinnners, RG; Burkart, JK; Malanchuk, M. (1980) Facilities for diesel exhaust studies. In: Health effects of diesel  
54 engine emissions: proceedings of an international symposium; December 1979. Pepelko, WE; Danner, RM;

1 Clarke, NA, eds. Cincinnati, OH: U.S. Environmental Protection Agency, Health Effects Research Laboratory; pp.  
2 681-697; EPA report no. EPA-600/9-80-057b. Available from: NTIS, Springfield, VA; PB81-173817.  
3

4 Hou, S; Lambert, B; Hemminki, K. (1995) Relationship between hprt mutant frequency, aromatic DNA adducts  
5 and genotypes for *GSTM1* and *NAT2* in bus maintenance workers. *Carcinogenesis* 16:1913-1917.  
6

7 Huisingsh, J; Bradow, R; Jungers, R; et al. (1978) Application of bioassay to the characterization of diesel particle  
8 emissions. In: Application of short-term bioassays in the fractionation and analysis of complex environmental  
9 mixtures: [proceedings of a symposium; February; Williamsburg, VA]. (Hollaender, A; Probststein, F; Welch, BL,  
10 eds. Environmental science research: v. 15.) Waters, MD; Nesnow, S; Huisingsh, JL; et al., eds. New York:  
11 Plenum Press; pp. 383-418.  
12

13 IARC (International Agency for Research on Cancer). (1989) Diesel and gasoline engine exhausts and some  
14 nitroarenes. (IARC monographs on the evaluation of carcinogenic risks to humans: v. 46). Lyon, France: World  
15 Health Organization; pp. 41-185.  
16

17 Ichinose, T; Yajima, Y; Nagashima, M; et al. (1997) Lung carcinogenesis and formation of 8-hydroxy-  
18 deoxyguanosine in mice by diesel exhaust particles. *Carcinogenesis* 18:185-192.  
19

20 Ishinishi, N; Koizumi, A; McClellan, RO; et al., eds. (1986) Carcinogenic and mutagenic effects of diesel engine  
21 exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel  
22 engines; July; Tsukuba Science City, Japan. (Developments in toxicology and environmental science: v. 13.)  
23 Amsterdam: Elsevier Science Publishers BV.  
24

25 Keane, MJ; Xing, SG; Harrison, JC; et al. (1991) Genotoxicity of diesel-exhaust particles dispersed in simulated  
26 pulmonary surfactant. *Mutat Res* 260:233-238.  
27

28 Lewtas, J. (1982) Mutagenic activity of diesel emissions. In: Toxicological effects of emissions from diesel engines:  
29 proceedings of the Environmental Protection Agency 1981 diesel emissions symposium; October 1981; Raleigh,  
30 NC. (Developments in toxicology and environmental science: v. 10.) Lewtas, J, ed. New York: Elsevier  
31 Biomedical; pp. 243-264.  
32

33 Lewtas, J. (1983) Evaluation of the mutagenicity and carcinogenicity of motor vehicle emissions in short-term  
34 bioassays. *Environ Health Perspect* 47:141-152.  
35

36 Li, AP; Dutcher, JS. (1983) Mutagenicity of mono-, di-, and tri-nitropyrenes in Chinese hamster ovary cells. *Mutat*  
37 *Res* 119:387-392.  
38

39 Li, AP; Royer, RE. (1982) Diesel-exhaust-particle extract enhancement of chemical-induced mutagenesis in  
40 cultured Chinese hamster ovary cells: possible interaction of diesel exhaust with environmental chemicals. *Mutat*  
41 *Res* 103:349-355.  
42

43 Liber, HL; Andon, BM; Hites, RA; et al. (1981) Diesel soot: mutation measurements in bacterial and human cells.  
44 *Environ Int* 5:281-284.  
45

46 Liberti, A; Cicciooli, P; Cecinato, A; et al. (1984) Determination of nitrated-polyaromatic hydrocarbons (nitro-  
47 PAHs) in environmental samples by high resolution chromatographic techniques. *J High Resolut Chromatogr*  
48 *Commun* 7:389-397.  
49

50 Liu, X; Keane, MJ; Zong, BZ; et al. (1996) Micronucleus formation in V79 cells treated with respirable silica  
51 dispersed in medium and simulated pulmonary surfactant. *Mutat Res* 361:89-94.  
52

53 Loft, S; Deng, XS; Tuo, J; et al. (1998) Experimental study of oxidative DNA damage. *Free Radical Res* 29:525-  
54 539.

1 Matsushita, H; Goto, S; Endo, O; et al. (1986) Mutagenicity of diesel exhaust and related chemicals. In:  
2 Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium  
3 on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. (Developments on  
4 toxicology and environmental science: v. 13.) Ishinishi, N; Koizumi, A; McClellan, RO; et al., eds. Amsterdam:  
5 Elsevier Science Publishers BV; pp. 103-118.  
6

7 Mitchell, AD; Evans, EL; Jotz, MM; et al. (1981) Mutagenic and carcinogenic potency of extracts of diesel and  
8 related environmental emissions: in vitro mutagenesis and DNA damage. *Environ Int* 5:393-401.  
9

10 Morimoto, K; Kitamura, M; Kondo, H; et al. (1986) Genotoxicity of diesel exhaust emissions in a battery of in-  
11 vitro short-term bioassays. In: Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the  
12 international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science  
13 City, Japan. (Developments in toxicology and environmental science: v. 13.) Ishinishi, N; Koizumi, A; McClellan,  
14 RO; et al., eds. Amsterdam: Elsevier Science Publishers BV; pp. 85-102.  
15

16 Nakagawa, R; Kitamori, S; Horikawa, K; et al. (1983) Identification of dinitropyrenes in diesel-exhaust particles:  
17 their probable presence as the major mutagens. *Mutat Res* 124:201-211.  
18

19 Nagashima, M; Kasai, H; Yokota, J; et al. (1995) Formation of an oxidative DNA damage, 8-  
20 hydroxydeoxyguanosine, in mouse lung DNA after intratracheal instillation of diesel exhaust particles and effects  
21 of high dietary fat and beta-carotene on this process. *Carcinogenesis* 16:1441-1445.  
22

23 Nehls, P; Seiler, F; Rehn, B; et al. (1997) Formation and persistence of 8-oxoguanine in rat lung cells as an  
24 important determinant in tumor formation following particle exposure. *Environ Health Perspect* 105(5):1291-1296.  
25

26 Nielsen, PS; Autrup, H. (1994) Diesel exhaust-related DNA adducts in garage workers. *Clin Chem* 40:1456-1458.  
27

28 Nielsen, PS; Andreassen, Å; Farmer, PB; et al. (1996) Biomonitoring of diesel-exhaust exposed workers. DNA and  
29 hemoglobin adducts and urinary 1-hydroxyproline as markers of exposure. *Toxicol Lett* 86:27-37.  
30

31 Nishioka, MG; Petersen, BA; Lewtas, J. (1982) Comparison of nitro-aromatic content and direct-acting  
32 mutagenicity of diesel emissions. In: Polynuclear aromatic hydrocarbons: physical and biological chemistry.  
33 Cooke, M; Dennis, AJ; Fisher, GL, eds. Columbus, OH: Battelle Press; pp. 603-613.  
34

35 Pepelko, WE; Peirano, WB. (1983) Health effects of exposure to diesel engine emissions: a summary of animal  
36 studies conducted by the U.S. Environmental Protection Agency's Health Effects Research Laboratories at  
37 Cincinnati, Ohio. *J Am Coll Toxicol* 2:253-306.  
38

39 Pereira, MA; Connor, TH; Meyne, J; et al. (1981a) Metaphase analysis, micronucleus assay and urinary  
40 mutagenicity assay of mice exposed to diesel emissions. *Environ Int* 5:435-438.  
41

42 Pereira, MA; Sabharwal, PS; Gordon, L; et al. (1981b) The effect of diesel exhaust on sperm-shape abnormalities  
43 in mice. *Environ Int* 5:459-460.  
44

45 Pereira, MA; Sabharwal, PS; Kaur, P; et al. (1981c) In vivo detection of mutagenic effects of diesel exhaust by  
46 short-term mammalian bioassays. *Environ Int* 5:439-443.  
47

48 Pereira, MA; McMillan, L; Kaur, P; et al. (1982) Effect of diesel exhaust emissions, particulates, and extract on  
49 sister chromatid exchange in transplacentally exposed fetal hamster liver. *Environ Mutagen* 4:215-220.  
50

51 Rannug, U; Sundvall, A; Westerholm, R; et al. (1983) Some aspects of mutagenicity testing of the particulate  
52 phase and the gas phase of diluted and undiluted automobile exhaust. *Environ Sci Res* 27:3-16.  
53

- 1 Rosenkranz, HS. (1993) Revisiting the role of mutagenesis in the induction of lung tumors in rats by diesel  
2 emissions. *Mutat Res* 303:91-95.  
3
- 4 Russell, LB; Generoso, WM; Oakberg, EF; et al. (1980) Tests for heritable effects induced by diesel exhaust in the  
5 mouse. Martin Marietta Energy Systems, Inc., Oak Ridge National Laboratory; report no. ORNL-5685.  
6
- 7 Salmeen, I; Durisin, AM; Prater, TJ; et al. (1982) Contribution of 1-nitropyrene to direct-acting Ames assay  
8 mutagenicities of diesel particulate extracts. *Mutat Res* 104:17-23.  
9
- 10 Schuetzle, D; Frazier, JA. (1986) Factors influencing the emission of vapor and particulate phase components from  
11 diesel engines. In: *Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international*  
12 *satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan.*  
13 *(Developments in toxicology and environmental science: v. 13.)* Ishinishi, N; Koizumi, A; McClellan, RO; et al.,  
14 eds. Amsterdam: Elsevier Science Publishers BV; pp. 41-63.  
15
- 16 Schuetzle, D; Lewtas, J. (1986) Bioassay-directed chemical analysis in environmental research. *Anal Chem*  
17 58:1060A-1076A.  
18
- 19 Schuetzle, D; Perez, JM. (1983) Factors influencing the emissions of nitrated-polynuclear aromatic hydrocarbons  
20 (nitro-PAH) from diesel engines. *J Air Pollut Control Assoc* 33:751-755.  
21
- 22 Schuler, RL; Niemeier, RW. (1981) A study of diesel emissions on *Drosophila*. *Environ Int* 5:431-434.  
23
- 24 Shirmamé-Moré, L. (1995) Genotoxicity of diesel emissions. Part I: Mutagenicity and other genetic effects. Diesel  
25 exhaust: a critical analysis of emissions, exposure, and health effects. A special report of the Institute's Diesel  
26 Working Group. Cambridge, MA: Health Effects Institute, pp. 222-242.  
27
- 28 Siak, JS; Chan, TL; Lees, PS. (1981) Diesel particulate extracts in bacterial test systems. *Environ Int* 5:243-248.  
29
- 30 Sjögren, M; Li, H; Banner, C; et al. (1996) Influence of physical and chemical characteristics of diesel fuels and  
31 exhaust emissions on biological effects of particle extracts: a multivariate statistical analysis of ten diesel fuels.  
32 *Chem Res Toxicol* 9:197-207.  
33
- 34 Smith, BA; Fullerton, NF; Aidoo, A; et al. (1993) DNA adduct formation in relation to lymphocyte mutations and  
35 lung tumor induction in F344 rats treated with the environmental pollutant 1,6- dinitropyrene. *Environ Health*  
36 *Perspect* 99:277-280.  
37
- 38 Tucker, JD; Xu, J; Stewart, J; et al. (1986) Detection of sister chromatid exchanges induced by volatile  
39 genotoxicants. *Teratogen Carcinogen Mutagen* 6:15-21.  
40
- 41 U.S. Environmental Protection Agency (EPA). (1980) Health effects of diesel engine emissions: proceedings of an  
42 international symposium. Cincinnati, OH: Office of Research and Development; EPA 600/9-80/057b.  
43
- 44 Wong, D; Mitchell, CE; Wolff, RK; et al. (1986) Identification of DNA damage as a result of exposure of rats to  
45 diesel engine exhaust. *Carcinogenesis* 7:1595-1597.  
46
- 47 Zhang, Z; Shen, HM; Zhang, QF; et al. (1999) Involvement of oxidative stress in crystalline silica-induced  
48 cytotoxicity and genotoxicity in rat alveolar macrophages. *Environ Res* 82:245-252.