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Environmental Protection
Agency

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Development
Washington DC 20460

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External Review Draft

EPA

# Health Assessment Document for Diesel Emissions

# Review Draft (Do Not Cite or Quote)

## Volume II of II

Notice

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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# 7. CARCINOGENICITY OF DIESEL EMISSIONS IN LABORATORY ANIMALS

## 7.1 INTRODUCTION

The particulate phase of diesel exhaust is composed of aggregates of carbon particles; the primary particle diameter ranges from 10 to 80 nm, and aggregates of these primary particles have mass median diameters averaging 0.2 to 0.3 $\mu$m (Vuk et al., 1976; Carpenter and Johnson, 1980), although some may approach 1.0 $\mu$m. A great variety of organic compounds, including polycyclic aromatic hydrocarbons (PAHs), are adsorbed to this carbon core (see Tables 2-6 and 2-8) and comprise 5 to 65% of the total particle mass (Cuddihy et al., 1984). Some of these organic compounds, such as benzo[*a*]pyrene (B[*a*]P), dinitropyrenes, and 1-nitropyrene, have received special attention regarding their carcinogenic and mutagenic potential. These organics may be strongly or weakly bound to the carbon core and represent varying amounts of the total particle mass. Qualitative and quantitative relationships for these organics depend on such variables as fuel composition, engine design, and engine operating conditions. Although less emphasis has been placed on the gaseous phase, potential carcinogens such as formaldehyde, acetaldehyde, and benzene, as well as lower molecular weight PAHs, may be also be present in this fraction.

The respirability of these particles and their associated organics provides a basis for health hazard concerns, and the reported mutagenicity (Huisingh et al., 1978) and skin papilloma induction (Kotin et al., 1955) of solvent extracts of diesel soot suggests a potential for carcinogenicity. Zamora et al. (1983) provided evidence that diesel exhaust particle extracts contained components that acted as weak tumor promoters in vitro. Recently, emphasis has been directed toward assessing the carcinogenic potential of whole and filtered diesel exhaust using whole-animal studies and understanding the mechanisms and implications of deposition, retention, and clearance of the particulate phase of diesel exhaust.

This chapter summarizes studies that assess the carcinogenic potential of diesel exhaust in laboratory animals. Experimental protocols for the inhalation studies usually consisted of exposure (usually chronic) to diluted exhaust in whole-body exposure chambers using rats, mice, and hamsters as model species. Some of these studies used both filtered (free of

particulate matter) diesel exhaust and unfiltered (whole) diesel exhaust to differentiate gaseous-phase effects from effects induced by the particulate matter and its adsorbed components. Inhalation exposure to particulate matter alone, however was not reported. Particulate matter concentrations in the diesel exhaust used in these studies ranged from 0.1 to 12 $mg/m^3$. Clean air (usually filtered) was used in the control exposures. Studies providing both positive, negative, or inconclusive findings have been reported. In this chapter, any indication of statistical significance implies that $p \leq 0.05$ was reported in the reviewed publications. The experimental protocols and exposure atmosphere characterizations are not described in detail here but may be found in Appendix A. A summary of the animal carcinogenicity studies and their results are presented in Table 7-1.

Also included are studies that assessed the carcinogenic and/or tumorigenic effects of diesel exhaust particles and solvent extracts of these particles following dermal application, sc injection, ip injection, or intratracheal instillation in rodents, as well as cocarcinogenicity studies. Individual chemicals present in the gaseous phase or adsorbed to the particle surface were not included in this review because adequate assessments of those of likely concern (i.e., formaldehyde, acetaldehyde, benzene, and PAHs) have been published in other health assessment documents.

## 7.2 INHALATION STUDIES

### 7.2.1 Rat Studies

Mauderly et al. (1987) provided data affirming the carcinogenicity of automotive diesel engine exhaust in F344/Crl rats following chronic inhalation exposure. Male and female rats were exposed to diesel engine exhaust at nominal particulate matter concentrations of 0.35 (n = 366), 3.5 (n = 367), or 7.1 (n = 364) $mg/m^3$ for 7 h/day, 5 days/week for up to 30 mo. Sham-exposed (n = 365) controls breathed filtered room air. A total of 230, 223, 221, and 227 of these rats (sham-exposed, low-, medium-, and high-exposure groups, respectively) were examined for lung tumors. These numbers included those animals that died or were euthanized during exposure and those that were terminated following 30 mo of exposure. The exhaust was generated by 1980 Model 5.7-L Oldsmobile V-8 engines operated through continuously repeating U.S. Federal Test Procedure (FTP) urban

## TABLE 7-1. SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration (mg/m³) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Adenocarcinoma + Squamous Cell Carcinomas | Squamous Cysts | All Tumors | |
| Mauderly et al. (1987) | Rat/F344 | M + F, 230[b] | Clean air | 0 | None | 7 h/day, | NA | (0) | (0.9) | (0) | (0.9) | |
| | | M + F, 223 | Whole exhaust | 0.35 | None | 5 days/week | | (0) | (1.3) | (0) | (1.3) | |
| | | M + F, 221 | Whole exhaust | 3.5 | None | up to | | (2.3) | (0.5) | (0.9) | (3.6)[c] | |
| | | M + F, 227 | Whole exhaust | 7.1 | None | 30 mo | | (0.4) | (7.5) | (4.9) | (12.8)[c] | |
| | | | | | | | | Adenomas | Carcinomas | Squamous Cell Tumors | All Tumors | |
| Heinrich et al. (1986a,b) | Rat/ Wistar | F, 96 | Clean air | 0 | None | 19 h/day, | NA | 0/96 (0) | 0/96 (0) | 0/96 (0) | 0/96 (0) | |
| | | F, 92 | Filtered exhaust | 0 | None | 5 days/week | | 0/92 (0) | 0/92 (0) | 0/92 (0) | 0/92 (0) | |
| Mohr et al. (1986) | | F, 95 | Whole exhaust | 4.0 | None | for up to 35 mo | | 8/95 (8.4) | 0/95 (0) | 9/95 (9.4) | 17/95 (17.8)[c] | |
| | | | | | | | | Adenomas | Adenocarcinoma | Squamous Cell Tumors | All Tumors | |
| Heinrich et al. (1986a,b) | Mouse/ NMRI | M + F, 84 | Clean air | 0 | None | 19 h/day, | NA | 9/84 (11) | 2/84 (2) | — | 11/84 (13) | |
| | | M + F, 93 | Filtered exhaust | 0 | None | 5 days/week | | 11/93 (12) | 18/93 (19)[c] | — | 29/93 (31)[c] | |
| | | M + F, 76 | Whole exhaust | 4.0 | None | for up to 30 mo | | 11/76 (15) | 13/76 (17)[c] | — | 24/76 (32)[c] | |
| | Hamsters/ Syrian | M + F, 96 | Clean air | 0 | None | 19 h/day, | NA | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |
| | | M + F, 96 | Filtered exhaust | 0 | None | 5 days/week | | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |
| | | M + F, 96 | Whole exhaust | 4.0 | None | for up to 30 mo | | 0/96 (0) | 0/96 (0) | 0/96 | 0/96 (0) | |
| | | | | | | | | | | Squamous Cell Carcinoma | All Lung Tumors | |
| Henrich et al. (1989a) | Rat/ Wistar | F, NS | Clean air | 0 | DpN[d] | 19 h/day, | NA | | | (4.4) | (84.8) | |
| | | F, NS | Whole exhaust | 4.2 | DpN[d] | 5 days/week | | | | (46.8)[c] | (83.0) | |
| | | F, NS | Filtered exhaust | 0 | DpN[d] | for 24 to | | | | (4.4) | (67.4) | |
| | | F, NS | Clean air | 0 | DpN[e] | 30 mo | | | | (16.7) | (93.8) | |
| | | F, NS | Whole exhaust | 4.2 | DpN[e] | | | | | (31.3)[c] | (89.6) | |
| | | F, NS | Filtered exhaust | 0 | DpN[e] | | | | | (14.6) | (89.6) | |



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## TABLE 7-1 (cont'd). SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration (mg/m$^3$) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Adenosquamous Carcinomas | Squamous Cell Carcinomas | All Tumors | |
| Takaki et al. (1988) Light-duty engine | Rat/F344 | M + F, 123 | Clean air | 0 | None | 16 h/day, 6 days/week, for up to 30 mo | NA | 1/23 (0.8) | 2/123 (1.6) | 1/23 (0.8) | 4/123 (3.3) | |
| | | M + F, 123 | Whole exhaust | 0.1 | None | | | 1/23 (0.8) | 1/23 (0.8) | 1/23 (0.8) | 3/123 (2.4) | |
| | | M + F, 125 | Whole exhaust | 0.4 | None | | | 1/25 (0.8) | 0/125 (0) | 0/125 (0) | 1/125 (0.8) | |
| | | M + F, 123 | Whole exhaust | 1.1 | None | | | 0/23 (0) | 5/123 (4.1) | 0/123 (0) | 5/123 (4.1) | |
| | | M + F, 124 | Whole exhaust | 2.3 | None | | | 1/24 (8.1) | 2/124 (1.6) | 0/124 (0) | 3/124 (2.4) | |
| | | | | | | | | Adenomas | Adenosquamous Carcinomas | Squamous Cell Carcinomas | All Tumors | |
| Ishinishi et al. (1988b) Heavy-duty engine | Rat/F344 | M + F, 123 | Clean air | 0 | None | 16 h/day, 6 days/week, for up to 30 mo | NA | 0/123 (0) | 1/123 (0.8) | 0/123 (0) | 1/123 (0.8) | |
| | | M + F, 123 | Whole exhaust | 0.5 | None | | | 0/123 (0) | 0/123 (0) | 1/123 (0.8) | 1/123 (0.8) | |
| | | M + F, 125 | Whole exhaust | 1.0 | None | | | 0/125 (0) | 0/125 (0) | 0/125 (0) | 0/125 (0) | |
| | | M + F, 123 | Whole exhaust | 1.8 | None | | | 0/123 (0) | 4/123 (3.3) | 0/123 (0) | 4/123 (3.3) | |
| | | M + F, 124 | Whole exhaust | 3.7 | None | | | 0/124 (0) | 6/124 (4.8) | 2/124 (1.6) | 8/124 (6.5)[c] | |
| | | | | 0 | | | | | | | | |
| | | | | 0 | | | | | | | | |
| | | | | 4.9 | | | | Adenomas | Adenocarcinoma and Adeno-Squamous Carcinoma | Large Cell and Squamous Cell Carcinomas | All Tumors | |
| Iwai et al. (1986) | Rat/F344 | F, 24 | Clean air | 0 | None | 8 h/day, 7 days/week, for 24 mo | NA | 1/22 (4.5) | 0/22 (0) | 0/22 (0) | 1/22 (4.5)[f] | |
| | | F, 24 | Filtered exhaust | 0 | None | | | 0/16 (0) | 0/16 (0) | 0/16 (0) | 0/16 (0) | |
| | | F, 24 | Whole exhaust | 4.9 | None | | | 3/19 (0) | 3/19 (15.8) | 2/19 (10.5) | 8/19 (42.1)[c,g] | |
| | | | | | | | | | Adenoma | Carcinoma | | |
| Takemoto et al. (1986) | Rat/F344 | F, 12 | Clean air | 0 | None | 4 h/day, 4 days/week, 18-24 mo | NA | | 0/12 (0) | 0/12 (0) | | |
| | | F, 21 | Clean air | 0 | DIPN[h] | | | | 10/21 (47.6) | 4/21 (19) | | |
| | | F, 15 | Whole exhaust | 2-4 | None | | | | 0/15 (0) | 0/15 (0) | | |
| | | F, 18 | Whole exhaust | 2-4 | DIPN[h] | | | | 12/18 (66.7) | 7/18 (38.9) | | |



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## TABLE 7-1 (cont'd). SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration (mg/m$^3$) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenoma | Adeno-Carcinoma | |
| Takemoto et al. (1986) (cont'd) | Mouse/ IRC | M + F, 45 | Clean air | 0 | None | 4 h/day, 4 days/week, for 19-28 mo | NA | 3/45 (6.7) | 1/45 (2.2) | |
| | | M + F, 69 | Whole exhaust | 2-4 | None | | | 6/69 (8.7) | 3/69 (4.3) | |
| | Mouse/ C57BL | M + F, 12 | Clean air | 0 | None | 4 h/day, 4 days/week for 19-28 mo | NA | 1/12 (8.3) | 0/12 (0) | |
| | | M + F, 38 | Whole exhaust | 2-4 | None | | | 8/38 (21.1) | 3/38 (7.9) | |
| | | | | | | | | Primary Lung Tumors | | |
| Brightwell et al. (1989) | Rat/344 | M + F, 260 | Clean air | 0 | None | 16 h/day, 5 days/week, for 24 mo | NA | 3/260 (1.2) | | Tumor incidence for all interim sacrifice periods |
| | | M + F, 144 | Filtered exhaust (medium exposure) | 0 | None | | | 0/144 (0) | | |
| | | M + F, 143 | Filtered exhaust (high exposure) | 0 | None | | | 0/143 (0) | | |
| | | M + F, 143 | Whole exhaust | 0.7 | None | | | 1/143 (0.7) | | ♀ 24/25 (96%) at 24-mo sacrifice ♂ 12/27 (44%) at 24-mo sacrifice |
| | | M + F, 144 | Whole exhaust | 2.2 | None | | | 14/144 (9.7)[c] | | |
| | | M + F, 143 | Whole exhaust | 6.6 | None | | | 55/143 (38.5)[c] | | |
| | | | | | | | | Primary Lung Tumors | | |
| | Hamster/ Syrian Golden | M + F, | Clean air | 0 | None | 16 hours/day, 5 days/week, for 24 mo | NA | 7/202 (3.5) | | Respiratory tract tumors not related to exhaust exposure for any of the groups |
| | | M + F, 202 | Clean air | 0 | DEN[j] | | | 4/104 (3.8) | | |
| | | M + F, 104 | Filtered exhaust (medium dose) | 0 | DEN[j] | | | 9/104 (8.7) | | |
| | | M + F, 104 | Filtered exhaust (high dose) | 0 | DEN[j] | | | 2/101 (2.0) | | |
| | | M + F, 101 | Whole exhaust | 0.7 | DEN[j] | | | 6/102 (5.9) | | |
| | | M + F, 102 | Whole exhaust | 2.2 | DEN[j] | | | 4/101 (3.9) | | |
| | | M + F, 101 | Whole exhaust | 6.6 | DEN[j] | | | 1/204 (0.5) | | |
| | | M + F, 204 | Filtered exhaust (high dose) | 0 | None | | | 0/203 (0) | | |
| | | M + F, 203 | Whole exhaust | 6.6 | None | | | | | |



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## TABLE 7-5 (cont'd). SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration (mg/m$^3$) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | |
| Karagianes et al. (1981) | Rat/Wistar | M, 40 | Clean air | 0 | None | 6 h/day, 5 days/week, for up to 20 mo | NA | 0/6 (0) | |
| | | M, 40 | Whole exhaust | 8.3 | None | | | 1/6 (16.6) | |
| | | | | | | | | Lung Tumors | |
| Orthoefer et al. (1981) (Pepelko and Peirano, 1983) | Mouse/ Strong A | M, 25 | Clean air | 0 | None | 20 h/day, 7 days/week, for 7 weeks | | 3/22 (13.6) | 0.13 Tumors/ mouse |
| | | | Whole exhaust | 6.4 | None | | 26 weeks | 7/19 (36.8) | 0.63 Tumors/ mouse |
| | | | Whole exhaust | 6.4 | UV irradiated | | 26 weeks | 6/22 (27.3) | 0.27 Tumors/ mouse |
| | | | | | | | | Lung Tumors | |
| | Mouse/ Jackson A | M + F, 40 | Clean air | 0 | None | 20 h/day, 7 days/week, for 8 weeks | 8 weeks | 16/36 (44.4) | 0.5 Tumors/ mouse |
| | | M + F, 40 | Whole exhaust | 6.4 | None | | 8 weeks | 11/34 (32.3) | 0.4 Tumors/ mouse |
| | Mouse/ Jackson A | F, 60 | Clean air | 0 | None | 20 h/day, 7 days/week, for approx. 7 mo. | | 4/58 (6.9) | 0.09 Tumors/ mouse |
| | | F, 60 | Clean air | 0 | Urethan[l] | | | 9/52 (17.3) | 0.25 Tumors/ mouse |
| | | F, 60 | Whole exhaust | 6.4 | None | | | 14/56 (25.0) | 0.32 Tumors/ mouse |
| | | F, 60 | Whole exhaust | 6.4 | Urethan[k] | | | 22/59 (37.3) | 0.39 Tumors/ mouse |
| | | M, 429 | Clean air | 0 | None | | | 73/403 (18.0) | 0.23 Tumors/ mouse |
| | | M, 430 | Whole exhaust | 6.4 | None | | | 66/368 (17.9) | 0.20 Tumors/ mouse |



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## TABLE 7-1 (cont'd). SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration (mg/m$^3$) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Carcinomas | All Tumors | |
| Pepelko and Peirano (1983) | Mouse/ Sencar | M + F, 260 | Clean air | 0 | None | Continuous for 15 mo | NA | (5.1) | (0.5) | (5.6) | |
| | | | Clean air | 0 | BHT[l] | | | (12.2) | (1.7) | (2.8) | |
| | | | Clean air | 0 | Urethan[k] | | | (8.1) | (0.9) | (9.0) | |
| | | | Whole exhaust | 12 | None | | | (10.2)[c] | (1.0) | (11.2)[c] | |
| | | | Whole exhaust | 12 | BHT[l] | | | (5.4) | (2.7) | (8.1) | |
| | | | Whole exhaust | 12 | Urethan[l] | | | (8.7) | (2.6) | (11.2) | |
| | | | | | | | | | All Tumors | | |
| | Mouse/ Strain A | M + F, 90 | Clean air | 0 | None | | NA | | 21/87 (24) | | 0.29 Tumors/ mouse |
| | | | Clean air | 0 | Exposure (darkness) | | | | 59/237 (24.9) | | 0.27 Tumors/ mouse |
| | | | Whole exhaust | 12 | Exposure | | | | 10/80 12.5) | | 0.14 |
| | | | Whole exhaust | 12 | (darkness) | | | | 22/250 (0.10) | | 0.10 |
| | | | Clean air | 0 | Urethan[m] | | | | 66/75 (88) | | 2.80 |
| | | | Whole exhaust | 12 | Urethan[m] | | | | 42/75 (0.95) | | 0.95 |
| | | | | | | | | | Broncho-alveolar Carcinoma | | |
| Kaplan et al. (1983) White et al. (1983) | Rat/F344 | M, 30 | Clean air | 0 | None | 20 h/day, 7 days/week, for up to 15 mo | 8 mo | | 0/30 (0) | | |
| | | M, 30 | Whole exhaust | 0.25 | None | | 8 mo | | 1/30 (3.3) | | |
| | | M, 30 | Whole exhaust | 0.75 | None | | 8 mo | | 3/30 (10.0) | | |
| | | M, 30 | Whole exhaust | 1.5 | None | | 8 mo | | 1/30 (3.3) | | |
| | | | | | | | | | Pulmonary Adenoma | | |
| | Mouse/ A/J | M, 388 | Clean air | 0 | None | 20 h/day, 7 days/week, for up to 8 mo | NA | | 130/388 (33.5) | | |
| | | M, 388 | Whole exhaust | 0.25 | None | | | | 131/388 (33.8) | | |
| | | M, 399 | Whole exhaust | 0.75 | None | | | | 109/399 (27.3) | | |
| | | M, 396 | Whole exhaust | 1.5 | None | | | | 99/396 (25.0) | | |
| | | | | | | | | | Pulmonary Adenomas | | |
| Kaplan et al. (1982) | Mouse/ A/J | M, 458 | Clean air | 0 | None | 20 h/day, 7 days/week, for 3 mo | 6 mo | | 144/458 (31.4) | | |
| | | M, 18 | Clean air | 0 | Urethan[k] | | | | 18/18 (100) | | |
| | | M, 485 | Whole exhaust | 1.5 | None | | | | 165/485 (34.2) | | |



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## TABLE 7-1 (cont'd). SUMMARY OF ANIMAL CARCINOGENICITY STUDIES

| Study | Species/ Strain | Sex/ Total Number | Exposure Atmosphere | Particle Concentration ($mg/m^3$) | Other Treatment | Exposure Protocol | Postexposure Observation | Tumor Type and Incidence (%)[a] | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Adenomas | Carcinomas | All Tumors | |
| Ishinishi et al. (1988b) | Rat/F344 | NS, 5 | Whole exhaust | 0.1 | None | 16 h/day, | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | |
| | | NS, 8 | Whole exhaust | 0.1 | None | 6 days/week, | 12 mo | 0/8 (0) | 0/8 (0) | 0/8 (0) | |
| | | NS, 11 | Whole exhaust | 0.1 | None | for 12 mo | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | |
| Light duty | | NS, 5 | Whole exhaust | 1.1 | None | | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | |
| | | NS, 9 | Whole exhaust | 1.1 | None | | 12 mo | 0/9 (0) | 0/9 (0) | 0/9 (0) | |
| | | NS, 11 | Whole exhaust | 1.1 | None | | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | |
| Heavy duty | | NS, 5 | Whole exhaust | 0.5 | None | 16 h/day, | 6 mo | 0/5 (0) | 0/5 (0) | 0/5 (0) | |
| | | NS, 9 | Whole exhaust | 0.5 | None | 6 days/week, | 12 mo | 0/9 (0) | 0/9 (0) | 0/9 (0) | |
| | | NS 11 | Whole exhaust | 0.5 | None | for 12 mo | 18 mo | 0/11 (0) | 0/11 (0) | 0/11 (0) | |
| | | NS, 5 | Whole exhaust | 1.8 | None | | 6 mo | 0/5 (0) | 0/5 (0) | 0/11 (0) | |
| | | NS, 6 | Whole exhaust | 1.8 | None | | 12 mo | 0/6 (0) | 0/6 (0) | 0/6 (0) | |
| | | NS, 13 | Whole exhaust | 1.8 | None | | 18 mo | 0/13 (0) | 1/13 (0) | 1/13 (0) | |
| Lewis et al. (1986) | Rat/F344 | M + F, 288[n] | Clean air | 0 | None | 7 h/day, | NA | No tumors | | 0/192 (0) | |
| | | | Whole exhaust | 2.0 | None | 5 days/week, 24 mo[o] | | | | 0/192 (0) | |

[a]Table values indicate number exposed/number with tumors (% animals with tumors).
[b]Number of animals examined for tumors.
[c]Significantly different from clean air controls.
[d]Diphenylnitrosamine; 6.25 mg/kg/week sc during first 25 weeks of exposure.
[e]Diphenylnitrasamine; 12.5 mg/kg/week sc during first 25 weeks of exposure.
[f]Splenic lymphomas also detected in controls (8.3%), filtered exhaust group (37.5%) and whole exhaust group (25%).
[g]5.3% incidence of large cell carcinomas.
[h]1 g/kg, ip 1/week for 3 weeks starting 1 mo into exposure.
[i]Includes adenomas, squamous cell carcinomas, adenocarcinomas, adeno squamous cell carcinoma, and mesotheliomas.
[j]4.5 mg/DEN/kg, sc, 3 days prior to start of inhalation exposure.
[k]Single ip dose 1 mg/kg at start of exposure.
[l]Butylated hydroxytoluene 300 mg/kg, ip for Week 1, 83 mg/kg for Week 2, and 150 mg/kg for Weeks 3 to 52.
[m]12 $mg/m^3$ from 12 weeks of age to termination of exposure. Prior exposure (in utero) and of parents was 6 $mg/m^3$.
[n]120-121 males and 71-72 females examined histologically.
[o]Not all animals were exposed for full term, at least 10 males were killed at 3-, 6-, and 12-mo exposure.

NS = Not specified.
NA = Not applicable.



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certification cycles. The engines were equipped with automatic transmissions connected to eddy-current dynamometers and flywheels simulating resistive and inertial loads of a midsize passenger car. The D-2 diesel control fuel (Phillips Chemical Co.) met U.S. Environmental Protection Agency (EPA) certification standards and contained approximately 30% aromatic hydrocarbons and 0.3% sulfur. Following passage through a standard automotive muffler and tail pipe, the exhaust was diluted 10:1 with filtered air in a dilution tunnel and serially diluted to the final concentrations. The primary dilution process was such that particle coagulation was retarded. Mokler et al. (1984) provided a detailed description of the exposure system. The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A. No exposure-related changes in body weight or life span were noted for any of the exposed animals nor were there any signs of overt toxicity. Collective lung tumor incidence was greater (z statistic, $p \leq 0.05$) in the high (7.1-mg/m$^3$) and medium (3.5-mg/m$^3$) exposure groups (12.8 and 3.6%, respectively) versus the control and low (0.35-mg/m$^3$) exposure groups (0.9 and 1.3%, respectively). Bronchoalveolar adenomas, adenocarcinomas, and squamous cysts (considered benign, except for two that were classified as squamous cell carcinomas because of the presence of less differentiated cells and invasion of blood and lymph vessels) were identified. Using the same statistical analysis of specific tumor types, adenocarcinoma plus squamous cell carcinoma and squamous cyst incidence was significantly greater in the high-exposure group, and the incidence of adenomas was significantly greater in the medium exposure group. A significant ($p < 0.001$) exposure-response relationship was obtained for tumor incidence relative to exposure concentration and lung burden of particulate matter (soot). These data are summarized in Table 7-1. A logistic regression model estimating tumor prevalence as a function of time, dose (lung burden of soot), and sex indicated a sharp increase in tumor prevalence for the high dose level at about 800 days after the commencement of exposure. A less pronounced, but definite, increase in prevalence with time was predicted for medium dose levels. Significant effects were not detected at the low concentration. The particulate matter burdens (mg per lung) of rats exposed to 0.35, 3.5, or 7.1 mg of soot/m$^3$ for 24 mo were 0.6, 11.5, and 20.8, respectively, and affirmed the greater than predicted accumulation that was the result of decreased particle clearance following high-exposure conditions.

In summary, this study demonstrated the pulmonary carcinogenicity of high concentrations of whole, diluted diesel exhaust in rats following chronic inhalation exposure. Additionally, increasing lung particle burden resulting from this high-level exposure from a decreased clearance was demonstrated. A logistic regression model presented by Mauderly et al. (1987) indicated that both lung particulate matter burden and exposure concentration may be useful for expressing exposure-effect relationships.

A series of studies was conducted at the Fraunhofer-Institute in which female SPF Wistar rats were exposed for 19 h/day, 5 days/week to both filtered and unfiltered (total) diesel exhaust at an average particulate matter concentration of 4.24 $mg/m^3$. Animals were exposed for a maximum of 2.5 years. The exposure system as described by Heinrich et al. (1986b), used a 40 kw 1.6-L diesel engine operated continuously under the U.S. 72 FTP driving cycle. The engines used European Reference Fuel with a sulfur content of 0.36%. Filtered exhaust was obtained by passing engine exhaust through a Luwa FP-65 HT 610 particle filter heated to 80 °C and a secondary series of filters (Luwa FP-85, Luwa NS-30, and Drager CH 63302) at room temperature. The filtered and unfiltered exhausts were each diluted 1:17 with filtered air and passed through respective 12-$m^3$ exposure chambers. Mass median aerodynamic diameter of the diesel exhaust particulate matter was $0.35 \pm 0.10$ $\mu m$ (mean $\pm$ SD). The gas-phase components of the diesel exhaust atmospheres are presented in Appendix A.

The effects of exposure to either filtered or unfiltered exhaust were described by Heinrich et al. (1986a) and Stöber (1986). Exposure to unfiltered exhaust resulted in eight bronchoalveolar adenomas and nine squamous cell tumors in 15 of 95 rats examined for a 15.8% tumor incidence. Although statistical analysis was not provided, the increase appears to be highly significant. In addition to the bronchio-alveolar adenomas and squamous cell tumors, there was a high incidence of bronchio-alveolar hyperplasia (99%) and metaplasia of the bronchio-alveolar epithelium (65%). No tumors were reported among female Wistar rats exposed to filtered exhaust (n = 92) or clean air (n = 96).

Mohr et al. (1986) provided a more detailed description of the lung lesions and tumors identified in Heinrich et al. (1986a,b) and Stöber (1986). Substantial alveolar deposition of carbonaceous particles was noted for rats exposed to the unfiltered diesel exhaust. Squamous metaplasia was observed in 65.3% of the rats breathing unfiltered diesel exhaust but not in

any of the control rats. Of the nine squamous cell tumors, one was characterized as a Grade I carcinoma (borderline atypia, few to moderate mitoses, and slight evidence of stromal invasion), whereas the remaining eight were classified as benign, keratinizing, cystic tumors.

The effect of chronic (19 h/day, 5 days/week, 2 to 2.5 years) diesel exhaust exposure on the tumor-inducing effect of dipentylnitrosamine (DPN) was examined using female Wistar rats (Heinrich et al., 1986a; Stöber, 1986; Heinrich et al., 1989a). Groups of rats (45 to 48 per group) were exposed to clean air or whole diesel exhaust (particle concentration of 4.24 mg/m$^3$, as described previously) and administered by subcutaneous injection 250 or 500 mg DPN/kg/week during the first 25 weeks of exposure. The total DPN dose administered equalled 6.25 or 12.5 g/kg of body weight. The concentrations of B[*a*]P, benzo[*e*]pyrene (B[*e*]P), and chrysene in the diesel exhaust were 13, 21, and 76 ng/m$^3$, respectively.

The overall tumor rate in the lungs of DPN-treated rats was not affected by the exposure to either filtered or whole diesel engine exhaust. However, when only pulmonary squamous cell carcinomas were considered, the exposure to whole diesel exhaust significantly ($p \leq 0.05$) increased the tumor incidence (Table 7-1). Conversely, the high level of nasal tumors induced by DPN was significantly decreased in the rats exposed to the diesel engine emissions.

Heinrich et al. (1986b) and Mohr et al. (1986) compared the effects of exposure to particles having only a minimal carbon core but a much greater concentration of PAHs than does diesel particulate matter. The desired exposure conditions were achieved by mixing coal oven flue gas with pyrolyzed pitch. The concentration of B[*a*]P and other PAHs/mg particulate matter was about three orders of magnitude greater than that of diesel exhaust. Female rats were exposed to the flue gas-pyrolyzed pitch for 16 h/day, 5 days/week at particle concentrations of 3 to 7 mg/m$^3$ for 22 mo, then held in clean air for up to an additional 12 mo. Of 116 animals exposed, 22 tumors were reported in 21 animals, for an incidence of 18.1%. One was a bronchiolo-alveolar adenoma, one was a bronchiolo-alveolar carcinoma, and 20 were squamous cell tumors. Among the latter, 16 were classified as benign keratinizing cystic tumors and four were classified as carcinomas. No tumors were

reported in 115 controls. The tumor incidence in this study was comparable to that reported previously for the diesel-exhaust-exposed animals.

In a recent, yet unpublished study, female Wistar rats were exposed for 18 h/day, 5 days/week for 10 or 20 mo to a carbon black (CB) aerosol at a mean concentration of 6.09 mg/m$^3$ (Heinrich, 1990). The purpose of this study was to compare the carcinogenicity of particles having no more than traces of PAHs with those of diesel exhaust. Groups of 72 animals each were exposed for either 10 mo and held an additional 20 mo in clean air or for 20 mo then held an additional 10 mo in clean air. Among the rats exposed for 10 mo, malignant tumors were seen in 14% and benign tumors in additional 3%. These included three animals with bronchio-alveolar adenocarcinoma, seven with squamous-cell carcinoma, one with an adenosquamous carcinoma and two with bronchiolo-alveolar adenoma. In the group exposed for 20 mo, the tumor incidence was 8%, but all were malignant (one animal with bronchiolo-alveolar adenocarcinoma and five with squamous-cell carcinoma. No tumors were seen in the control groups. Another study in which the comparative tumorigenicity of CB and diesel exhaust was conducted at the Inhalation Toxicology Research Institute (Mauderly et al., 1991; Nikula et al., 1991, 1994). In this study, F344 rats were exposed to either CB or diesel exhaust for 16 h/day, 5 days/week to particle concentrations of 2.5 or 6.5 mg/m$^3$ for up to 24 mo. Controls were exposed to clean air. Preliminary results show that both diesel exhaust and carbon black are pulmonary carcinogens under the exposure conditions of the study (Nikula et al., 1991). Nineteen animals in both the high-exposure diesel exhaust (HDE) and the high-exposure carbon black (HCB) groups exhibited primary lung tumors (grossly observed or suspected and histologically confirmed). For the HDE group there were 8 squamous cysts, 4 adenomas, 37 adenocarcinomas, and 2 squamous cell carcinomas, and for the HCB group there were 17 squamous cysts, 6 adenomas, 26 adenocarcinomas, 2 squamous cell carcinomas, and 1 "other" malignant tumor. Primary lung tumors were observed in 11 rats of the low-exposure diesel exhaust (LDE) group and four rats of the low exposure carbon black (LCB) group. For the LDE group there were 3 squamous cell cysts, 2 adenomas, 7 adenocarcinomas, and 3 squamous cell carcinomas, and for the LCB group there were 7 squamous cell cysts, 1 adenocarcinoma, 2 squamous cell carcinomas, and 1 "other" malignant tumor.

In analyzing the studies of Heinrich et al. (1986a,b), Heinrich (1990), Mohr et al. (1986), and Stöber (1986), it must be noted that the incidence of lung tumors occurring following exposure to whole diesel exhaust, coal oven flue gas, or CB (15.8, 18.1, and 8 to 17%, respectively) was very similar. This occurred despite the fact that the PAH content of the PAH-enriched pyrolyzed pitch was more than three orders of magnitude greater than that of diesel exhaust; CB on the other hand, had only traces of PAHs. Based on these findings, the organic fraction is not the sole cause of tumor induction by diesel exhaust. This issue is discussed further in Chapter 10.

A long-term inhalation study (Ishinishi et al., 1988b; Takaki et al., 1989) examined the effects of emissions from light-duty (LD) and heavy-duty (HD) diesel engines on male and female Fischer 344/Jcl rats. The LD engines were 1.8-L, 4-cylinder, swirl-chamber-type power plants, and the HD engines were 11-L, 6-cylinder, direct-injection-type power plants. The engines were connected to eddy-current dynamometers and operated at 1,200 rpm (LD engines) and 1,700 rpm (HD engines). Nippon Oil Co. JIS No. 1 or No. 2 diesel fuel was used. The 30-mo whole-body exposure protocol (16 h/day, 6 days/week) employed diesel exhaust particle concentrations of 0, 0.5, 1, 1.8, or 3.7 mg/m$^3$ from HD engines and 0, 0.1, 0.4, 1.1, or 2.3 mg/m$^3$ from LD engines. The B[*a*]P concentrations were reported as 4.4 and 2.8 $\mu$g/g of particulate matter, and 1-nitropyrene concentrations were 57.1 and 15.3 $\mu$g/g of particulate matter for the LD and HD engines, respectively. An analysis of gas-phase components is presented in Appendix A. The animals inhaled the exhaust emissions from 1700 to 0900 hours. Sixty-four male rats and 59 to 61 female rats from each exposure group were evaluated for carcinogenicity.

For the experiments using the LD series engines, the highest incidence of hyperplastic lesions plus tumors (72.6%) was seen in the highest exposure (2.3 mg/m$^3$) group. However, this high value was the result of the 70% incidence of hyperplastic lesions; the incidence of adenomas was only 0.8% and that of carcinomas 1.6%. Hyperplastic lesion incidence was considerably lower for the lower-exposure groups (9.7, 4.8, 3.3, and 3.3% for the 1.1-, 0.4-, and 0.1-mg/m$^3$ and control groups, respectively). The incidence of adenomas and carcinomas, combining males and females, was not significantly different among exposure groups (2.4, 4.0, 0.8, 2.4, and 3.3% for the 2.3-, 1.1-, 0.4-, and 0.1-mg/m$^3$ groups and the controls, respectively).

For the experiments employing the HD series engines, the total incidence of hyperplastic lesions, adenomas, and carcinomas was highest (26.6%) in the 3.7-mg/m$^3$ exposure group. The incidence of adenomas plus carcinomas for males and females combined equalled 6.5, 3.3, 0, 0.8, and 0.8% at 3.7, 1.8, 1, and 0.4 mg/m$^3$ and for controls, respectively. A statistically significant difference was reported between the 3.7 mg/m$^3$ and the control groups for the HD series engines. A progressive dose-response relationship was not demonstrated. Tumor incidence data for this experiment are presented in Table 7-1.

The Ishinishi et al. (1988b) study also included recovery tests in which rats exposed to whole diesel exhaust (particle concentration of 0.1 or 1.1 mg/m$^3$ for the LD engine and 0.5 or 1.8 mg/m$^3$ for the HD engine) for 12 mo were examined for lung tumors following 6-, 12-, or 18-mo recovery periods in clean air. The incidences of neoplastic lesions were low, and pulmonary soot burden was lower than for animals continuously exposed to whole diesel exhaust and not provided a recovery period. The only carcinoma observed was in a rat examined 12 mo following exposure to exhaust (1.8 mg/m$^3$) from the HD engine.

Iwai et al. (1986) also examined the long-term effects of diesel exhaust inhalation on female F344 rats. The exhaust was generated by a 2.4-L displacement truck engine. The exhaust was diluted 10:1 with clean air at 20 to 25 °C and 50% relative humidity. The engines were operated at 1,000 rpm with an 80% engine load. These operating conditions were found to produce exhaust with the highest particle concentration and lowest $NO_2$ and $SO_2$ content. For those chambers using filtered exhaust, proximally installed high-efficiency particulate air (HEPA) filters were employed. Three groups of 24 rats each were exposed to unfiltered diesel exhaust, filtered diesel exhaust, or filtered room air for 8 h/day, 7 days/week for 24 mo. Particle concentration was 4.9 mg/m$^3$ for unfiltered exhaust. Concentrations of gas-phase exhaust components were 30.9 ppm $NO_x$, 1.8 ppm $NO_2$, 13.1 ppm $SO_2$, and 7.0 ppm CO.

No lung tumors were found in the 2-year control (filtered room air) rats, although one adenoma was noted in a 30-mo control rat, providing a spontaneous tumor incidence of 4.5%. No lung tumors were observed for rats exposed to filtered diesel exhaust. Four of 14 rats exposed to unfiltered diesel exhaust for 2 years developed lung tumors, two of these being malignant. Five rats of this 2-year exposure group were subsequently placed in clean

room air for 3 to 6 mo and four eventually (time not specified) exhibited lung tumors (three malignancies). Thus, the lung tumor incidence for total tumors was 42.1% (8/19) and 26.3% (5/19) for malignant tumors in rats exposed to whole diesel exhaust. The tumor types identified were adenomas (3/19), adenocarcinomas (1/19), adenosquamous carcinoma (2/19), squamous carcinoma (1/19), and large-cell carcinoma (1/19). The lung tumor incidence in rats exposed to whole diesel exhaust was significantly greater than that of controls ($p \leq 0.01$). Tumor data are summarized in Table 7-1. Malignant splenic lymphomas were detected in 37.5% of the rats in the filtered exhaust group and in 25.0% of the rats in the unfiltered exhaust group, these values being significantly ($p \leq 0.05$) greater than the 8.2% incidence noted for the control rats. The study demonstrates production of lung cancer in rats following 2-year exposure to unfiltered diesel exhaust. Additionally, the occurrence of splenic, malignant lymphomas occurred during exposure to both filtered and unfiltered diesel exhaust. This is the only report to date of tumor induction at an extrarespiratory site.

A chronic (up to 24 mo) inhalation exposure study by Takemoto et al. (1986) was conducted to determine the effects of diesel exhaust, di-isopropanol-nitrosamine (DIPN), and diesel exhaust following DIPN treatment to female F344/Jcl rats. One month after initiation of inhalation exposures, DIPN was administered ip at 1 mg/kg weekly for 3 weeks to clean air and diesel exposed groups of rats. Uninjected groups were also exposed to clean air and diesel exhaust. The treatment protocol consisted of exposure to diesel exhaust for 4 h/day, 4 days/week. The diesel exhaust was generated by a 269-cc displacement engine operated at an idle state (1,600 rpm). Concentrations of the gas-phase components of the exhaust are presented in Appendix A. The particle concentration of the diesel exhaust in the exposure chamber was 2 to 4 mg/m$^3$. Benzo[*a*]pyrene and 1-nitropyrene concentrations were 0.85 and 93 $\mu$g/g of particles, respectively.

In the Takemoto et al. (1986) study, no lung tumors were reported in either uninjected controls or diesel-exposed animals. Among injected animals autopsied at 12 to 17 mo, 2 adenomas were reported in 8 rats exposed to clean air compared with 12 adenomas and 3 adenocarcinomas in 18 diesel-exposed rats. Among injected rats autopsied at 18 to 24 mo, 10 adenomas and 4 adenocarcinomas were seen in 21 animals exposed to clean air compared with 12 adenomas and 7 adenocarcinomas in 18 diesel-exposed rats. According to the authors, the incidence of malignant tumors was not significantly increased in either of the

diesel exhaust-exposed groups when compared with the appropriate control group. Tumor incidence data for the various treatment protocols are presented in Table 7-1. It was also noted that the diesel engine employed in this study was originally used as an electrical generator and that its operating characteristics (not specified) were different from those for a diesel-powered automobile; however, the investigators deemed it suitable for assessing the of effects diesel emissions.

Brightwell et al. (1986, 1989) studied the effects of filtered and unfiltered diesel exhaust on male and female F344 rats. The diesel exhaust was generated by a 1.5-L Volkswagen engine that was computer-operated according to the U.S. 72 FTP driving cycle. The engine emissions were diluted by conditioned air delivered at 800 $m^3/h$ to produce the high-exposure (6.6 $mg/m^3$) diesel exhaust atmosphere. Further dilutions of 1:3 and 1:9 produced the medium- (2.2 $mg/m^3$) and low- (0.7 $mg/m^3$) exposure atmospheres. Filtered diesel exhaust was generated by a similar engine. The CO and $NO_x$ concentrations (mean $\pm$ SD) were 32 $\pm$ 11 ppm and 8 $\pm$ 1 ppm for the unfiltered diesel exhaust (high-exposure concentration chamber) and 32 $\pm$ 11 and 8 $\pm$ 1 for the filtered diesel exhaust. The inhalation exposures were conducted overnight to provide five 16-h periods per week for 2 years; surviving animals were maintained for an additional 6 mo.

For males and females combined, a 9.7% (14/144) and 38.5% (55/143) incidence of primary lung tumors occurred in F344 rats following exposure to 2.2 and 6.6 mg of diesel soot/$m^3$, respectively (Table 7-1). The tumor incidence in the 0.7-$mg/m^3$ exposure group was 0.7% (1/144) and that of controls was 1.2% (3/260). Diesel-exhaust-induced tumor incidence in rats was dose-related and higher in females than males (Table 7-1). These data included animals sacrificed at the interim periods (6, 12, 18, and 24 mo) therefore, the tumor incidence does not accurately reflect the effects of long-term exposure to the diesel exhaust atmospheres. When tumor incidence is expressed relative to the specific interim sacrifice period, a lung tumor incidence of 96% (24/25), 76% (19/25) of which were malignant, was reported for female rats in the high-dose group exposed for 24 mo and held in clean air for the remainder of their lives. For male rats in the same group, the tumor incidence equalled 44% (12/27), of which 37% (10/27) were malignant. It was also noted that many of the animals exhibiting tumors had more than one tumor, often representing multiple histological types. The types of tumors identified in the rats exposed to diesel exhaust included

adenomas, squamous cell carcinomas, adenocarcinomas, mixed adenoma/adenocarcinomas, and mesotheliomas. Similar to other studies, the tumor incidence in rats occurred during exposure to whole exhaust rather than filtered exhaust. It must be noted, however, that the exposure during darkness (when increased activity would result in greater respiratory exchange and greater inhaled dose) could account, in part, for the high response reported for the rats.

Karagianes et al. (1981) exposed male Wistar rats (40 per group) to diesel engine exhaust diluted to a particle concentration of 8.3 ($\pm$ 2.0) mg/m$^3$, room air, diesel engine exhaust (8.3 mg/m$^3$) plus low-concentration coal dust (5.8 mg/m$^3$), low-concentration coal dust only (6.6 mg/m$^3$), or high-concentration coal dust (14.9 mg/m$^3$) 6 h/day, 5 days/week for up to 20 mo. The exhaust-generating system and exposure atmosphere characteristics are presented in Appendix A. The type of engine used (3-cylinder, 43-bhp diesel) is normally used in mining situations and was connected to an electric generator and operated at varying loads and speeds to simulate operating conditions in an occupational situation. To control the CO concentration at 50 ppm, the exhaust was diluted 35:1 with compressed air.

One bronchiolar adenoma was detected in the group exposed to diesel exhaust alone and one in the rats receiving combined exposures. No lung tumors were reported in controls or following exposure to either high or low concentrations of coal dust. The equivocal response may have been caused by the relatively short exposure durations (20 mo). In the Mauderly et al. (1987) study, by comparison, most of the tumors were detected in rats exposed for more than 24 mo.

Lewis et al. (1986) also examined the effects of inhalation exposure of diesel exhaust and/or coal dust on tumorigenesis on F344 rats. Groups of 216 male and 72 female rats were exposed to clean air, whole diesel exhaust (2 mg soot/m$^3$), coal dust (2 mg/m$^3$), or diesel exhaust plus coal dust (1 mg/m$^3$ of each) for 7 h/day, 5 days/week for up to 24 mo. Groups of 10 or more males were sacrificed at intermediate intervals (3, 6, and 12 mo). The diesel exhaust was produced by a 7.0-L, four-cycle, water-cooled Caterpillar Model 3304 engine using No. 2 diesel fuel ($<$0.5% sulfur by mass). The exhaust was passed through a Wagner water scrubber, which lowered the exhaust temperature and quenched engine backfire. An analysis of the exposure atmospheres is presented in Appendix A.

Histological examination was performed on 120 to 121 male and 71 to 72 female rats terminated after 24 mo of exposure. No specific tumor information was provided other than that the exhaust exposure did not significantly affect the tumor incidence. There was no postexposure period, which may explain, in part, the lack of significant tumor induction. The particulate matter concentration was also less than the effective dose in several of the other studies.

General Motors Research Laboratories sponsored chronic inhalation studies using male Fischer 344 rats exposed to diesel exhaust particle concentrations of 0.25, 0.75, or 1.5 mg/m$^3$ (Kaplan et al., 1983; White et al., 1983). The exposure protocol for this study conducted at the Southwest Research Institute (SWRI) was 20 h/day, 7 days/week for 9 to 15 mo. Some animals were sacrificed following completion of exposure, whereas others were returned to clean-air atmospheres for an additional 8 mo. Control animals received clean air. Exhaust was generated by 5.7-L Oldsmobile engines (four different engines used throughout the experiment) operated at a steady speed and load simulating a 40-mph driving speed of a full-size passenger car. Details of the exhaust-generating system and exposure atmosphere are presented in Appendix A.

Five instances of bronchoalveolar carcinoma were observed out of 90 rats exposed to diesel exhaust for 15 mo and held an additional 8 mo in clean air. These included one tumor in the 0.25-mg/m$^3$ group, three in the 0.75-mg/m$^3$ group, and one in the 1.5-mg/m$^3$ group. Rats kept in clean air chambers for 23 mo did not exhibit any carcinomas. No tumors were observed in any of the 180 rats exposed to diesel exhaust for 9 or 15 mo without a recovery period or in the respective controls for these groups. Although the increase in tumor incidences in the groups exposed for 15 mo and held an additional 8 mo in clean air were not statistically significant, they are suggestive of an effect because the background incidence for this specific lesion in this strain of rat is low.

### 7.2.2 Mouse Studies

Heinrich et al. (1986a) and Stöber (1986), as part of a larger study, also evaluated the effects of diesel exhaust in mice. Details of the exposure conditions reported by Heinrich et al. (1986b) are given in Appendix A. Following lifetime (19 h/day, 5 days/week, for a maximum of 120 weeks) exposure to filtered (n = 93) and unfiltered (n = 76) diesel exhaust

(4.2.4 mg/m$^3$), female NMRI mice exhibited a total lung tumor incidence of adenomas and adenocarcinomas combined of 31% (filtered) and 32% (unfiltered), respectively. Tumor incidences reported for control mice (n = 84) equalled 11% for adenomas and adenocarcinomas combined. The effects are more dramatic when the incidences of only malignant tumors (adenocarcinomas) are considered, 2.4% for controls, 19% for filtered exhaust, and 17% for unfiltered exhaust. This is the only reported study in which filtered exhaust resulted in a definitive tumorigenic response in the lungs of mice. These data are summarized in Table 7-1.

As part of the same study, groups of 64 female NMRI mice of 8 to 10 weeks of age were dosed weekly with either 50 or 100 $\mu$g B[*a*]P intratracheally for 20 or 10 weeks, respectively, for a total dose of 1 mg. Another group received 50 $\mu$g dibenz[*a,h*]-anthracene (DBA) intratracheally for 10 weeks. Additional groups of 96 newborn mice received one sc injection of 5 or 10 $\mu$g of DBA between 24 and 48 h after birth. The animals were concomitantly exposed to either diesel exhaust or clean air. The mice receiving intratracheal instillations were observed throughout their lifespan but the newborn mice were sacrificed after 6 mo. Although the chemical treatments resulted in large increases in lung tumor incidence, exposure to diesel exhaust did not enhance this effect and in some cases even resulted in inhibition. For example, lung tumor rates in clean air mice treated with 20 instillations of B[*a*]P equalled 71% compared with 41% for mice similarly instilled but exposed to diesel exhaust. The decrease resulted from a smaller number of adenocarcinomas, whereas the adenoma incidence remained unchanged. The high dose of DBA injected into newborn mice also resulted in a greater tumor incidence in mice exposed to clean air (81%) than in the diesel exposed group (63%). Effects of the other treatments were apparently not inhibited by diesel exhaust exposure, although complete incidence data were not reported. The authors did not speculate on the reasons for this unexpected effect.

Takemoto et al. (1986) reported the effects of inhaled diesel exhaust (2 to 4 mg/m$^3$, 4 h/day, 4 days/week, for up to 28 mo) in ICR and C57BL mice exposed from birth. Details of the exposure conditions are presented in Appendix A. Among male and female ICR mice autopsied at 13 to 18 mo, 4 adenomas and 1 adenocarcinoma were detected in 34 diesel exhaust-exposed mice compared with 3 adenomas among 38 controls. Among animals autopsied at 19 to 28 mo, 6 adenomas and 3 adenocarcinomas were seen in

22 exposed animals, compared with 3 adenomas and one adenocarcinoma in 22 controls. Among combined male and female C57BL mice autopsied at 13 to 18 mo, 4 adenomas and 2 adenocarcinomas were detected in 79 animals autopsied compared with none in 19 unexposed animals. Among males and females autopsied at 19 to 28 mo, 8 adenomas and 3 adenocarcinomas were detected in 71 exposed animals compared with one adenoma among 32 controls. No significant increases in either adenoma or adenocarcinoma incidences were reported for either strain of exposed mice. Although not tested by the authors, the combined incidence of adenomas and adenocarcinomas (11/71) in male and female C57BL mice exposed to diesel exhaust for 19 to 28 mo versus that found in controls (1/32), however, appears to be a significant increase. Although the results are not definitive, there is the strong suggestion of an effect, especially since the C57BL strain has a low background lung tumor incidence. See Table 7-1 for details of tumor incidence.

Pepelko and Peirano (1983) summarized a series of studies on the health effects of diesel emissions in mice. Exhaust was provided by two Nissan CN 6-33, 6-cylinder, 3.24-L diesel engines coupled to a Chrysler A-272 automatic transmission and Eaton model 758-DG dynamometer. Details of the exhaust generating system and the exposure atmosphere are presented in Appendix A. Sixty-day pilot studies were conducted at a 1:14 dilution, providing particle concentrations of 6 $mg/m^3$. The engines were operated using the Modified California Cycle. These 20-h/day, 7-day/week pilot studies using rats, cats, guinea pigs, and mice produced decreases in weight gain and food consumption. Therefore, at the beginning of the long-term studies, exposure time was reduced to 8 h/day, 7 days/week at an exhaust particle concentration of 6 $mg/m^3$. During the final 12 mo of exposure, however, the particle concentration was increased to 12 $mg/m^3$. For the chronic studies, the engines were operated using the Federal Short Cycle.

Pepelko and Peirano (1983) described a two-generation study using Sencar mice exposed to diesel exhaust alone or treated with either tumor initiators or promoters. Male and female parent generation mice were exposed to diesel exhaust at a particle concentration of 6 $mg/m^3$ prior to (from weaning to sexual maturity) and throughout mating. The dams continued exposure through gestation, birth, and weaning. Groups of offspring (130 males and 130 females) received ip injections of either butylated hydroxytoluene (BHT) (300 mg/kg for week 1, 83 mg/kg for week 2, and 150 mg/kg from week 3 to 1 year), a single ip

injection of 1 mg urethan at 6 weeks of age, or no injections. The exhaust exposure was increased to a particle concentration of 12 mg/m$^3$ when the offspring were 12 weeks of age and was maintained until termination of the experiment when the mice were 15 mo old.

The incidence of pulmonary adenomas (16.3%) was significantly increased in the noninjected female mice exposed to diesel exhaust, compared with 6.3% in clean air controls. The incidence in males and females combined was 10.2% in 205 animals examined compared with 5.1% in 205 clean air controls. This difference was also significant. The incidence of carcinomas was not affected by exhaust exposure in either sex. Exhaust exposure reduced the adenoma incidence in female mice receiving BHT (3.9 versus 16.7%). The response to BHT in males, or urethan in both sexes was unaffected by diesel exposure. These results provided the earliest evidence for cancer induction following inhalation exposure to diesel exhaust. The limited response may well have been influenced by the relatively early sacrifice times of the mice. On the other hand, an increase in the sensitivity of the study, allowing detection of tumors at 15 mo, may have been the result of exposure from conception. It is interesting to note that in this study diesel exposure appeared to inhibit effects of tumor promotion, whereas Stöber (1986) reported diesel exposure inhibition of complete carcinogens. These data are summarized in Table 7-1.

A series of inhalation studies, using strain A mice, was conducted by Orthoefer et al. (1981). In assays with the strain A, mice are usually given a series of sc injections with the test agent; they are then sacrificed at about 9 mo of age and examined for lung tumors. In the present series inhalation exposure was substituted. In the current series, groups of 25 male Strong A mice were exposed to irradiated (to simulate chemical reactions induced by sunlight) or nonirradiated diesel exhaust (6 mg/m$^3$) for 20 h/day, 7 days/week for 7 weeks. Additional groups of 40 Jackson A (20 of each sex) were exposed similarly to either clean air or diesel exhaust then held in clean air until sacrificed at 9 mo of age. No tumorigenic effects were detected at nine months of age. Further studies were conducted in which male A/Strong mice were exposed 8 h/day, 7 days/week until sacrifice (approximately 300 at 9 mo of age and approximately 100 at 12 mo of age). With the exception of those treated with urethan, the number of tumors per mouse did not exceed historical control levels in any of the studies. Exposure to diesel exhaust, however, significantly inhibited the tumorigenic effects of the 5-mg urethan treatment. Results are listed in Table 7-1.

Kaplan et al. (1982) also reported the effects of diesel exposure in strain A mice. Groups of male strain A/J mice were exposed for 20 h/day, 7 days/week for 90 days, and held until 9 mo of age. Experimental conditions are described in Appendix A. Briefly, the animals were exposed to diesel exhaust at particle concentrations of 0, 0.25, 0.75, or 1.5 mg/m$^3$. Controls were exposed to clean air. Among 458 controls and 485 exposed animals, tumors were detected in 31.4% of those breathing clean air versus 34.2% of those exposed to diesel exhaust. The mean number of tumors per mouse also failed to show significant differences.

In a follow-up study, strain A mice were exposed to diesel exhaust for 8 mo (Kaplan et al., 1983; White et al., 1983). After exposure to the highest exhaust concentration (1.5 mg/m$^3$), the percentage of mice with pulmonary adenomas and the mean number of tumors per mouse were significantly less ($p < 0.05$) than for controls (25.0 versus 33.5% and 0.30 $\pm$ 0.02 [S.E.] versus 0.42 $\pm$ 0.03 [S.E.]) (Table 7-1).

### 7.2.3 Hamster Studies

Heinrich et al. (1982) examined the effects of diesel exhaust exposure on the tumor frequency in female Syrian golden hamsters pretreated with the tumor initiators DBA or diethylnitrosamine (DEN). At the time of this work, it was presumed that traditional inhalation exposure experiments would not result in definitive tumor formation; thus a tumor initiation animal model was used. Groups of 48 to 72 animals were exposed to clean air, whole diesel exhaust at a mean particle concentration of 3.9 mg/m$^3$, or filtered diesel exhaust with either no further treatment or administered DBA (intratracheal instillations of 0.1 mg/week for 20 weeks), DEN (1.5 or 4.5 mg/kg sc) or pyrene (intratracheal instillations of 0.1 mg/week for 20 weeks), the last serving as a noncarcinogenic PAH control. Inhalation exposures were conducted 7 to 8 h/day, 5 days/week for 2 years. The exhaust was produced by a 2.4-L Daimler-Benz engine operated at 2,400 rpm.

Only two hamsters exhibited lung tumors, both having died during the exposure period. One occurred in a hamster receiving DBA and exposed to filtered diesel exhaust for 75 weeks; the other occurred in a hamster receiving DEN and exposed to whole diesel exhaust for 67 weeks. Compared with corresponding treatment groups, there was a higher incidence of adenomatous proliferative changes in the lungs of hamsters exposed to whole

diesel exhaust. Hamsters exposed to filtered diesel exhaust also showed a greater incidence of adenomatous proliferative changes than did those of the respective clean air exposure groups. The incidence of proliferative changes in the lungs of hamsters receiving DEN or DBA was greater than for those groups not treated with the initiators. Although not definitive, this study provided information suggesting the possible involvement of whole diesel exhaust and filtered diesel exhaust in producing histologic alterations in the lungs of hamsters, though no increases in tumors were observed.

In a more recent study, Syrian hamsters were exposed 19 h/day, 5 days/week for a lifetime to diesel exhaust diluted to a particulate matter concentration of 4.24 mg/m$^3$ (Heinrich et al., 1986a; Stöber, 1986). Details of the exposure conditions are reported in Appendix A. Ninety-six animals per group were exposed to clean air, whole exhaust, or filtered exhaust. Additional groups were treated with DEN (4.5 mg/kg, sc) or B[*a*]P (20 doses of 0.25 mg intratracheal instillation) and exposed to the three experimental atmospheres. No lung tumors were seen in uninjected clean-air or in either diesel exhaust-exposed group. Initial treatment with DEN or B[*a*]P resulted in lung tumor incidences of only 10 and 2%, respectively, which were not significantly changed by exposure to diesel exhaust.

Heinrich et al. (1989b) reported results of experiments assessing the effects of DEN and diesel exhaust exposure in combination. Hamsters were exposed to exhaust from a Daimler-Benz 2.4-L engine operated at a constant load of about 15 kW and at a uniform speed of 2,000 rpm. The exhaust was diluted to an exhaust-clean-air ratio of about 1:13, resulting in a mean particle concentration of 3.75 mg/m$^3$. The animals were exposed 19 h/day, 5 days/week beginning at noon each day, under a 12-h light cycle, starting at 0700 hours. DEN (3 or 6 mg/kg) was given as a single sc injection 2 weeks from the start of exposure to groups of 40 male and 40 female Syrian golden hamsters exposed to whole diesel exhaust, filtered diesel exhaust, or clean air. Groups were also exposed to the exhaust without DEN or to only clean air. Exposures were conducted in chambers maintained at 22 to 24 °C and 40 to 60% relative humidity for up to 18 mo. Surviving hamsters were maintained in clean air for up to an additional 6 mo. The concentrations of B[*a*]P and B[*e*]P in the whole exhaust atmospheres were 37.5 and 61.9 ng/m$^3$, respectively.

No lung tumors were detected in any of the hamsters of any of the treatment groups. A nasal carcinoma was detected in a female hamster treated with DEN (6 mg/kg) and exposed to filtered exhaust. A tracheal carcinoma was detected in a male hamster exposed to whole diesel exhaust and receiving DEN (3 mg/kg), and a laryngeal carcinoma observed in a male hamster receiving DEN (6 mg/kg) and exposed to whole diesel exhaust. Exposure of male hamsters to whole or filtered diesel exhaust alone did not result in a significant increase in the tumors relative to clean air controls. Male hamsters receiving 6 mg DEN/kg plus whole diesel exhaust exposure and dying before or after the 50% survival date, however, did show an increase in tumor rate compared with DEN-treated animals exposed to clean air. Using life-table analysis, a significant ($p < 0.05$) exposure-related increase in tumor rate was noted for this group (40.0 versus 7.0% for filtered exhaust + DEN and 7.0% for clean air + DEN). No upper respiratory tract tumors were detected in clean-air controls, or filtered-exhaust-exposed groups that did not receive the DEN treatment.

In summary, diesel exhaust alone did not induce an increase in lung tumors in hamsters of either sex. Diesel exhaust did significantly enhance the tumorigenic effects of DEN in males injected with 6 mg DEN/kg but not in females or in either sex given the 3 mg/kg dose. The cocarcinogenic effects of diesel with DEN therefore appear to be equivocal.

Brightwell et al. (1986, 1989) studied the effects of filtered or unfiltered diesel exhaust on male and female Syrian golden hamsters. Groups of 52 males and 52 females received no injections or sc injections of 4.5 mg DEN/kg 3 days before the start of exposure. The animals were 6 to 8 weeks old at the start of exposure to diesel exhaust at particle concentrations of 0.7, 2.2, or 6.6 mg/m$^3$. They were exposed 16 h/day, 5 days/week for a total of 2 years and then sacrificed. Exposure conditions are described in Appendix A. Although the DEN-pretreated hamsters exhibited an increase in tracheal papillomas in all treatment groups when compared with non-DEN pretreated hamsters, there was no statistically significant (t-test) relationship between tumor incidence and exhaust exposure. As noted in International Agency for Research on Cancer (1989), however, the reporting of tumor incidence and survival was incomplete.

### 7.2.4 Monkey Studies

Fifteen male cynomolgus monkeys were exposed to diesel exhaust (2 mg/m$^3$) for 7 h/day, 5 days/week for 24 mo (Lewis et al., 1986). The same numbers of animals were also exposed to coal dust (2 mg/m$^3$), diesel exhaust plus coal dust (1 mg/m$^3$ for each component), or filtered air. Details of exposure conditions were listed previously in the description of the Lewis et al. (1986) study with rats (Appendix A).

None of the monkeys exposed to diesel exhaust exhibited a significantly increased incidence of preneoplastic or neoplastic lesions. It should be noted, however, that the 24-mo time frame employed in this study may not have allowed the manifestation of tumors in primates, because this duration is only a small fraction of the monkeys expected life span.

## 7.3 LUNG IMPLANTATION OR INTRATRACHEAL INSTILLATION STUDIES

### 7.3.1 Rat Studies

Grimmer et al. (1987), using female Osborne Mendel rats (35 per treatment group), provided evidence that the PAHs in diesel exhaust that consist of four or more rings have a carcinogenic potential. Condensate was obtained from the whole exhaust of a 3.0-L passenger car diesel engine connected to a dynamometer, the operation of which simulated city traffic driving conditions. This condensate was separated by liquid-liquid distribution into hydrophilic and hydrophobic fractions representing 25 and 75% of the total condensate, respectively. The hydrophilic, hydrophobic, or reconstituted hydrophobic fractions were surgically implanted into the lungs of the rats. Untreated controls, vehicle (beeswax/trioctanoin) controls, and positive (B[*a*]P) controls were also included in the protocol (Table 7-2). Results of the various treatments are presented in Table 7-2. Fraction IIb (made up of PAHs with four to seven rings), which accounted for only 0.8% of the total weight of diesel exhaust condensate, produced the highest incidence of lung carcinomas following implantation into the rat lungs. A carcinoma incidence of 17.1% was observed following implantation of 0.21 mg IIb/rat, whereas the nitro-PAH fraction (IId) at 0.18 mg/rat accounted for only a 2.8% carcinoma incidence. Hydrophilic fractions of the diesel exhaust particulate extracts, vehicle (beeswax/trioctanoin) controls, and untreated

**TABLE 7-2. TUMOR INCIDENCE AND SURVIVAL TIME OF RATS TREATED WITH FRACTIONS FROM DIESEL EXHAUST CONDENSATE (35 RATS/GROUP)**

| Material Portion by Weight (%) | Dose (mg) | Median Survival Time in Weeks (range) | Number of Carcinomas[a] | Number of Adenomas[b] | Carcinoma Incidence (%) |
|---|---|---|---|---|---|
| Hydrophilic fraction(I) (25) | 6.70 | 97(24-139) | 0 | 1 | 0 |
| Hydrophobic fraction (II) (75) | 20.00 | 99(50-139) | 5 | 0 | 14.2 |
| Nonaromatics + | | | | | |
| PAC 2 + 3 rings (IIa) (72) | 19.22 | 103(25-140) | 0 | 1 | 0 |
| PAH 4 to 7 rings (IIb) (0.8) | 0.21 | 102(50-140) | 6 | 0 | 17.1 |
| Polar PAC (IIc) (1.1) | 0.29 | 97(44-138) | 0 | 0 | 0 |
| Nitro-PAH (IId) (0.7) | 0.19 | 106(32-135) | 1 | 0 | 2.8 |
| Reconstituted hydrophobics (Ia), b, c, d) (74.5) | 19.91 | 93(46-136) | 7 | 1 | 20.0 |
| Control, unrelated | | 110(23-138) | 0 | 0 | 0 |
| Control (beeswax/trioctanoin) | | 103(51-136) | 0 | 1 | 0 |
| Benzo[*a*]pyrene | 0.3 | 69(41-135) | 27 | 0 | 77.1 |
| | 0.1 | 98(22-134) | 11 | 0 | 31.4 |
| | 0.03 | 97(32-135) | 3 | 0 | 8.6 |

[a]Squamous cell carcinoma.
[b]Bronchiolar/alveolar adenoma.

Source: Adapted from Grimmer et al. (1987).



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controls failed to exhibit carcinoma formation. Administration of all hydrophobic fractions (IIa-d) produced a carcinoma incidence (20%), similar to the summed incidence of fraction IIb (17.1%) and IId (2.8%). The B[*a*]P positive controls (0.03, 0.1, and 0.3 mg/rat) yielded a carcinoma incidence of 8.6, 31.4, and 77.1%, respectively. The study showed that the tumorigenic agents were primarily 4- to 7-ring PAHs and, to a lesser extent, nitroaromatics. However, these studies demonstrated that simultaneous administration of various PAH compounds resulted in a varying of the tumorigenic effect, thereby implying that the tumorigenic potency of PAH mixtures may not depend on any one individual PAH. This study did not provide any information regarding the bioavailability of the particle-associated PAHs that might be responsible for carcinogenicity.

Kawabata et al. (1986) compared the effects of activated carbon and diesel exhaust on lung tumor formation. One group of 59 F344 rats was intratracheally instilled with diesel particles (1 mg/week for 10 weeks). A second group of 31 rats was instilled with the same dosing regime of activated carbon. Twenty-seven rats received only the solvent (buffered saline with 0.05% Tween 80), whereas 53 rats were uninjected. Rats dying after 18 mo were autopsied. All animals surviving 30 mo or more postinstillation were sacrificed and evaluated for histopathology. Among 42 animals exposed to diesel particulate matter surviving 18 mo or more, tumors were reported in 31, including 20 malignancies. In the subgroup surviving for 30 mo, tumors were detected in 19 of 20 animals, including 10 malignancies. Among the rats exposed to activated carbon, the incidence of lung tumors equalled 11 of 23 autopsied, with 7 cases of malignancy. Data for those dying between 18 and 30 mo and those sacrificed at 30 mo were not reported separately. Statistical analysis indicated that activated carbon induced a significant increase in lung tumor incidence compared with no tumors in 50 uninjected controls and one tumor in 23 solvent-injected controls. The tumor incidence increase was significant in the diesel exposed group, and was significantly greater than the increase in the carbon exposed group. This study provides evidence for the carcinogenicity of diesel particulate matter. It also shows, as did Heinrich (1990) for inhalation exposure, that particles lacking in organic constituents can also induce tumors.

### 7.3.2 Syrian Hamster Studies

Kunitake et al. (1986) and Ishinishi et al. (1988a) conducted a study in which total doses of 1.5, 7.5, or 15 mg of a dichloromethane extract of diesel exhaust was instilled intratracheally over 15 weeks into male Syrian hamsters that were then held for their lifetimes. The tumor incidence of 2.3% (1/44), 0% (0/56), and 1.7% (1/59) for the high-, medium-, and low-dose groups, respectively, did not differ significantly from the 1.7% (1/56) reported for controls. Addition of 7.5 mg of B[*a*]P to an exhaust extract dose of 1.5 mg resulted in a total tumor incidence of 91.2% and malignant tumor incidence of 88%. Benzo[*a*]pyrene (7.5 mg over 15 weeks) alone produced a tumor incidence rate of 88.2% (85% of these being malignant), which was not significantly different from the exhaust extract + B[*a*]P group. Intratracheal administration of 0.03 $\mu$g B[*a*]P, the equivalent content in 15 mg of exhaust extract, failed to cause a significant increase in tumors in rats. This study demonstrated a lack of detectable interaction between exhaust extract and B[*a*]P, the failure of exhaust extract to induce carcinogenesis, and the propensity for respiratory tract carcinogenesis following intratracheal instillation of high doses of B[*a*]P. For studies using the exhaust extract, some concern must be registered regarding the known differences in chemical composition between exhaust extract and whole diesel exhaust. As with all intratracheal instillation protocols, DE lacks the complement of volatile chemicals found in whole diesel exhaust.

The effects on hamsters of intratracheally instilled whole diesel exhaust suspension, diesel particles with $Fe_2O_3$, or diesel particle extract with $Fe_2O_3$ as the carrier were studied by Shefner et al. (1982). The diesel exhaust component in each of the treatments was administered at concentrations of 1.25, 2.5, or 5.0 mg/week for 15 weeks to groups of 50 male Syrian Golden hamsters. The total volume instilled was 3.0 mL (0.2 mL/week for 15 weeks). The diesel particles and the dichloromethane extracts were suspended in physiological saline with gelatin (0.5% w/v), gum arabic (0.5% w/v), and propylene glycol (10% by volume). The $Fe_2O_3$ concentration, when used, was 1.25 mg/0.2 mL of suspension. Controls received vehicle and, where appropriate, carrier particles ($Fe_2O_3$) without the exhaust component. Two replicates of the experiments were performed. Adenomatous hyperplasia was reported to be most severe in those animals treated with the diesel exhaust particles or the diesel exhaust particles plus $Fe_2O_3$ particles and least severe in

those animals receiving the diesel particle extract plus $Fe_2O_3$. Of the two lung adenomas detected microscopically, one was in a high-dose diesel-particle-treated animal and the other was in a high-dose diesel extract suspension-treated animal. Although lung damage was increased by instillation of diesel particles, there was no evidence of tumorigenicity.

## 7.4 SUBCUTANEOUS AND INTRAPERITONEAL INJECTION STUDIES

### 7.4.1 Mouse Studies

In addition to inhalation studies, Orthoefer et al. (1981) also tested the effects of ip injections of diesel exhaust particulate matter on male Strong A mice. Three groups of 30 mice were injected with 0.1 mL of a suspension (particles in distilled water) containing 47, 117, or 235 $\mu$g of diesel exhaust particles collected from Fluoropore filters in the inhalation exposure chambers. The exposure system and exposure atmosphere are described in Appendix A. Vehicle controls received injections of particle suspension made up of particulate matter from control exposure filters, positive controls received 20 mg of urethan, and negative controls received no injections. Injections were made three times weekly for 8 weeks, resulting in a total diesel particle dose of 1.1, 2.8, and 5.6 mg for the low-, medium-, and high-dose groups, respectively, and 20 mg of urethan for the positive control group. These animals were sacrificed after 26 weeks and examined for lung tumors. For the low-, medium-, and high-diesel exhaust particle dose groups, the tumor incidence was 2/30, 10/30, and 8/30, respectively. The incidence among urethan-treated animals (positive controls) was 100% (29/29), with multiple tumors per animal. The tumor incidence for the diesel exhaust-treated animals did not differ significantly from that of vehicle controls (8/30) or negative controls (7/28). The numbers of tumors per mouse was also unaffected by treatment.

In further studies conducted by Orthoefer et al. (1981), an attempt was made to compare the potency of diesel exhaust with those of other environmental pollutants. Male and female Strain A mice were injected ip three times weekly for 8 weeks with diesel emission particles, particle extracts, or various environmental mixtures of known carcinogenicity, including cigarette smoke condensate, coke oven emissions, and roofing tar

emissions. Injection of urethan or dimethylsulfoxide (DMSO) served as positive or vehicle controls, respectively. In addition to particulate matter from the Nissan diesel previously described (Section 7.2.2), an 8-cylinder Oldsmobile engine operated at the equivalent of 40 mph was also used for comparison of emission effects from different makes and models of diesel engine. The mice were sacrificed at 9 mo of age and their lungs examined for histopathological changes. The only significant findings, other than for positive controls, were small increases in numbers of lung adenomas per mouse in male mice injected with Nissan diesel engine exhaust extract and in female mice injected with coke oven extract. Furthermore, the increase in the extract-treated mice was significant only in comparison with uninjected controls, (not injected ones) and did not occur when the experiment was repeated. Despite the use of a strain of mouse known to be sensitive to tumor induction, the overall findings of this study were negative. The authors provided several possible explanations for these findings, the most likely of which were (1) the carcinogens that were present were very weak or (2) the concentrations of the active components reaching the lungs was insufficient to produce positive results.

Kunitake et al. (1986) conducted studies using an extract of exhaust obtained from a HD, 1983, MMC M-6D22P, 11-L V-6 engine. Five sc injections of exhaust extract (500 mg/kg per injection) resulted in a significant ($p < 0.01$) increase in subcutaneous tumors for female C57Bl mice (5/22 [22.7%] versus 0/38 among controls). Five sc doses of exhaust extract of 10, 25, 30, 100, or 200 mg/kg failed to produce a significant increase in tumor incidence. One of 12 female ICR mice (8.3%) and 4 of 12 male ICR mice (33.3%) developed malignant lymphomas following neonatal sc administration of 10 mg of exhaust extract per mouse. The increase in malignant lymphoma incidence for the male mice was statistically significant at ($p < 0.05$) compared with an incidence of 2/14 (14.3%) among controls. Treatment of either sex with 2.5 or 5 mg of exhaust extract per mouse did not result in statistically significant increases in tumor incidence.

Additional studies using particulate matter extract from LD (1.8-L, 4-cylinder) as well as HD engines with female ICR and nude mice (BALB/c/cA/JCL-nu) were also reported (Kunitake et al., 1988). Groups of 30 ICR and nude mice each were given a single sc injection of 10 mg HD extract, 10 mg HD + 50 $\mu$g 12-O-tetradecanoylphorbol 13-acetate (TPA), 10 mg LD extract + 50 $\mu$g TPA, or 50 $\mu$g TPA. No malignant tumors or

papillomas were observed. One papillomatous lesion was observed in an ICR mouse receiving LD extract + TPA, and acanthosis was observed in one nude mouse receiving only TPA.

In what appears to be an extension of the Kunitake et al. (1986) sc injection studies, Takemoto et al. (1988) presented additional data for subcutaneously administered extract of diesel exhaust from HD and LD diesel engines. In this report, the extracts were administered to 5-week-old and neonatal (<24-h-old) C57Bl mice of both sexes. Exhaust particle extract from HD or LD engines was administered weekly to the 5-week-old mice for 5 weeks at doses of 10, 25, 50, 100, 200, or 500 mg/kg, with group sizes ranging from 15 to 54 animals. After 20 weeks, comparison with a control group indicated a significant increase in the incidence of subcutaneous tumors for the 500-mg/kg HD group (5 of 22 mice [22.7%], $p < 0.01$), the 100 mg/kg LD group (6 of 32 [18.8%], $p < 0.01$), and the 500 mg/kg LD group (7 of 32 [21.9%], $p < 0.01$) in the adult mouse experiments. The tumors were characterized as malignant fibrous histiocytomas. No tumors were observed in other organs. The neonates were given single doses of 2.5, 5, or 10 mg-extract subcutaneously within 24 h of birth. There was a significantly higher incidence of malignant lymphomas in males receiving 10 mg of HD extract and of lung tumors for males given 2.5 mg HD extract and for males given 5 mg and females given 10 mg LD extract. A dose-related trend that was not significant was observed for the incidences of liver tumors for both the HD- and LD-treated neonatal mice. The incidence of mammary tumors in female mice and multiple-organ tumors in male mice was also greater for some extract-treated mice but was not dose related. The report concluded that LD particulate extract showed greater carcinogenicity than did HD particulate extract.

## 7.5 DERMAL STUDIES

### 7.5.1 Mouse Studies

In one of the earliest studies of diesel emissions, the effects of dermal application of extract from diesel exhaust particles was examined by Kotin et al. (1955). Acetone extracts were prepared from the exhaust soot of a diesel engine (type and size not provided) operated at warm-up mode and under load. These extracts were applied dermally, three times weekly

to male and female C57BL and Strain A mice. Results of these experiments are summarized in Table 7-3. In the initial experiments using 52 (12 male, 40 female) C57BL mice treated with exhaust extract from an engine operated in a warm-up mode, two papillomas were detected after 13 mo. Four tumors in 8 surviving of 50 exposed male Strain A mice treated with exhaust extract from an engine operated under full load were detected 16 mo after the start of treatment. For female Strain A mice treated with extract from an engine operated under full load, 17 tumors were detected in 20 of 25 mice surviving longer than 13 mo. This provided a significantly increased tumor incidence of 85%. Carcinomas as well as papillomas were seen, but the numbers were not reported.

Depass et al. (1982) examined the potential of diesel exhaust particles and dichloromethane extracts of diesel exhaust particles to act as complete carcinogens, carcinogen initiators, or carcinogen promoters. In skin-painting studies, the exhaust material was obtained from an Oldsmobile 5.7-L diesel engine operated under constant load at 65 km/h. The exhaust particles were collected at a temperature of 100 °C. Groups of 40 C3H/HeJ mice were used because of their low spontaneous tumor incidence. For the complete carcinogenesis experiments, diesel exhaust particles were applied as a 5 or 10% suspension in acetone. Dichloromethane extract was applied as 5, 10, 25, or 50% suspensions. Negative controls received acetone, and positive controls received 0.2% B[*a*]P. For tumor-promotion experiments, a single application of 1.5% B[*a*]P was followed by repeated applications of 10% diesel particle suspension, 50% diesel particle extract, acetone only (vehicle control), 0.0001% phorbol 12-myristate 13-acetate (PMA) as a positive promoter control, or no treatment (negative control). For the tumor-initiation studies, a single initiating dose of 10% diesel particle suspension, 50% diesel particle extract, acetone, or PMA was followed by repeated applications of 0.0001% PMA. Following 8 mo of treatment, the PMA dose in the initiation and promotion studies was increased to 0.01%. Animals were treated three times per week in the complete carcinogenesis and initiation experiments and five times per week in promotion experiments. All test compounds were applied to a shaved area on the back of the mouse.

In the complete carcinogenesis experiments, one mouse receiving the high-dose (50%) suspension of extract developed a squamous cell carcinoma after 714 days of treatment. Tumor incidence in the B[*a*]P group was 100% and no tumors were observed in any of the

**TABLE 7-3. TUMORIGENIC EFFECTS OF DERMAL APPLICATION OF ACETONE EXTRACTS OF DIESEL EXHAUST**

| Number of Animals | Strain/Sex | Sample Material | Time to First Tumor (months) | Survivors at Time of First Tumor | Total Tumors | Duration of Experiment (months) |
|---|---|---|---|---|---|---|
| 52 | C57/40 F<br>C57/12 M | Extract of particulate material obtained during warm-up | 13 | 33 | 2 | 22 |
| 50 | Strain A/M | Extract of particulate material obtained during full load | 15 | 8 | 4 | 23 |
| 25 | Strain A/F | Extract of particulate material obtained during full load | 13 | 20 | 17 | 17 |

Source: Kotin et al. (1955).



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other groups. For the promotion studies, squamous cell carcinomas with pulmonary metastases were identified in one mouse of the 50% diesel exhaust particle extract group, and one in the 25% extract group. Another mouse in the 25% extract group developed a grossly diagnosed papilloma. Nineteen positive control mice had tumors (11 papillomas, 8 carcinomas). No tumors were observed for any of the other treatment groups. For the initiation studies, three tumors (two papillomas and one carcinoma) were identified in the group receiving diesel particle suspension and three tumors (two papillomas and one fibrosarcoma) were found in the diesel exhaust particle extract group. These findings were reported to be statistically insignificant using the Breslow and Mantel-Cox tests.

The data from this study indicated that diesel exhaust particles and dichloromethane extracts of these particles are not effective with regard to tumor promotion or initiation. Although these findings were not consistent with those of Kotin et al. (1955) (Table 7-3), the occurrence of a single carcinoma in a strain known to have an extremely low spontaneous tumor incidence may be of importance. Furthermore, a comparison between studies employing different strains of mice with varying spontaneous tumor incidences may result in erroneous assumptions.

Nesnow et al. (1982) studied the formation of dermal papillomas and carcinomas following dermal application of dichloromethane extracts from coke oven emissions, roofing tar, diesel engine exhaust, and gasoline engine exhaust. Diesel exhaust from five different engines including a preproduction Nissan 220C, a 5.7-L Oldsmobile, a prototype VW Turbo Rabbit, a Mercedes 300D, and a HD Caterpillar 3304 were used for various phases of the study. Male and female Sencar mice (40 per group) were used for tumor-initiation, tumor-promotion, and complete carcinogenesis studies. For the tumor-initiation experiments, the diesel exhaust extracts were topically applied in single doses of 100, 500, 1,000 or 2,000 $\mu$g/mouse. The high dose (10,000 $\mu$g/mouse) was applied in five daily doses of 2,000 $\mu$g. One week later, 2 $\mu$g of the tumor promoter tetradecanoylphorbol acetate (TPA) was applied topically twice weekly. The tumor-promotion experiments used mice treated with 50.5 $\mu$g of B[*a*]P followed by weekly (twice weekly for high dose) topical applications (at the aforementioned doses) of the extracts. For the complete carcinogenesis experiments, the test extracts were applied weekly (twice weekly for the high doses) for 50 to 52 weeks.

Only extracts from the Nissan, Oldsmobile, and Caterpillar engines were used in the complete carcinogenesis experiments.

In the tumor-initiation studies, both B[*a*]P alone and the Nissan engine exhaust extract followed by TPA treatment produced a significant increase in tumor (dermal papillomas) incidence at 7 to 8 weeks postapplication. By 15 weeks, the tumor incidence was greater than 90% for both groups. No significant carcinoma formation was noted for mice in the tumor-initiation experiments following exposure to the exhaust extracts of the other diesel engines, although the Oldsmobile engine exhaust extract at 2.0 mg/mouse did produce a 40% papilloma incidence in male mice at 6 mo. This effect was, however, not dose dependent.

Benzo[*a*]pyrene (50.5 μg/week), coke oven extract (at 1.0, 2.0, or 4.0 mg/week), and the highest dose of roofing tar extract (4.0 mg/week) all tested positive for complete carcinogenesis activity. Exhaust extracts from only the Nissan, Oldsmobile, and Caterpillar engines were tested for complete carcinogenic potential, and all three proved to be negative using the Sencar mouse assay.

The results of the dermal application experiments by Nesnow et al. (1982) are presented in Table 7-4. The tumor initiation-promotion assay was considered positive if a dose-dependent response was obtained and if at least two doses provided a papilloma-per-mouse value that was three times or greater than that of the background value. Based on these criteria, only emissions from the Nissan were considered positive. Tumor initiation and complete carcinogenesis assays required that at least one dose produce a tumor incidence of at least 20%. None of the diesel exhaust samples yielded positive results based on this criterion.

Kunitake et al. (1986, 1988) evaluated the effects of a dichloromethane extract of diesel exhaust particulate matter obtained from a 1983 MMC M-6D22P, 11-L V-6 engine. An acetone solution was applied in 10 doses every other day, followed by promotion with 2.5 μg of TPA, three times weekly for 25 weeks. Exposure groups received a total dose of 0.5, 5, 15, or 45 mg of extract. Papillomas were reported in 2 of 50 animals examined in the 45-mg exposure group and 1/48 in the 15-mg group compared with 0/50 among controls. Differences, however, were not statistically significant.

## TABLE 7-4. DERMAL TUMORIGENIC AND CARCINOGENIC EFFECTS OF VARIOUS EMISSION EXTRACTS

| Sample | Tumor Initiation | | Complete Carcinogenesis | Tumor Promotion |
|---|---|---|---|---|
| | Papillomas[a] | Carcinomas[b] | Carcinomas[b] | Papilomas[a] |
| Benzo[*a*]pyrene | +/+[c] | +/+ | +/+ | +/+ |
| Topside coke oven | +/+ | -/+ | ND[d] | ND |
| Coke oven main | +/+ | +/+ | +/+ | +/+ |
| Roofing tar | +/+ | +/+ | +/+ | +/+ |
| Nissan | +/+ | +/+ | -/- | ND |
| Oldsmobile | +/+ | -/- | -/- | ND |
| VW Rabbit | +/+ | -/- | I[e] | ND |
| Mercedes | +/- | -/- | ND | ND |
| Caterpillar | -/- | -/- | -/- | ND |
| Residential furnace | -/- | -/- | ND | ND |
| Mustang | +/+ | -/+ | ND | ND |

[a]Scored at 6 mo.
[b]Cumulative score at 1 year.
[c]Male/female.
[d]ND = Not determined.
[e]I = Incomplete.

Source: Nesnow et al. (1982).



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## 7.6 SUMMARY AND CONCLUSIONS OF ANIMAL CARCINOGENICITY STUDIES

As early as 1955, Kotin et al. (1955) provided evidence for tumorigenicity and carcinogenicity of acetone extracts of diesel exhaust following dermal application and also provided data suggesting a difference in this potential depending on engine operating mode. Until the early 1980s, no chronic studies assessing inhalation of diesel exhaust, the relevant mode for human exposure, had been reported. Since then, inhalation studies have been emphasized. Studies employing rats and an experimental protocol including long-term exposure at high exposure concentrations (up to 8 mg/m$^3$), resulting in large lung particle loads and a postexposure observation period, were generally positive in demonstrating diesel exhaust-induced increases in tumorigenicity. The highest incidences of tumors were reported by Brightwell et al. (1986). Among female rats exposed for 24 mo and held for their lifetimes, tumors were detected in 24/25 animals. This study points out the probable cumulative effects of high exposure concentration (6.6 mg/m$^3$), lengthy daily exposures (16 h/day), exposure in the dark resulting in a probable increase in ventilation and thereby particle intake, and maintenance of the animals for their lifetimes. In two other major studies, Heinrich et al. (1986a) and Mauderly et al. (1987), significant but lower lung tumor incidences were reported at the high-dose levels, 15.8 and 12.8%, respectively. Although exposure concentrations differed, 7 mg/m$^3$ for Mauderly et al. versus 4 mg/m$^3$ for Heinrich et al., the longer daily exposure periods in the Heinrich et al. study, 19 h versus 7 h, would probably result in only slightly differing intakes. Ishinishi et al. (1988a,b) reported a 6.5% incidence of lung tumors in rats exposed to a concentration of 4 mg/m$^3$ particulate matter from a HD engine. In this study, although the concentration was relatively low, duration and length of daily exposure was long (16 h/day for 30 mo). Iwai et al. (1986) reported an increased lung tumor incidence (4/14) in Fischer rats exposed 8 h/day, 7 days/week for 24 mo to a particle concentration of 4.9 mg/m$^3$. Four of five held in clean air an additional 3 to 6 mo, however, also developed tumors pointing out again the importance of a long study duration. Iwai et al. (1986) reported the only diesel exhaust inhalation-induced tumor increase at a nonrespiratory site (splenic lymphoma).

Low exposure concentrations and/or short exposure durations were generally used in the negative studies (Karagianes et al., 1981; Lewis et al., 1986; White et al., 1983;

Takemoto et al., 1986). The lowest particle concentrations resulting in significant positive effects in rats were in the range of 2 to 3 mg/m$^3$.

Inhalation of diesel exhaust induced significant increases in lung tumors in female NMRI mice (Heinrich et al., 1986a; Stöber, 1986) and in female Sencar mice (Pepelko and Peirano, 1983). An apparent increase was also seen in female C57BL mice (Takemoto et al., 1986). In a series of short-term inhalation studies using Strain A, mice no increases in lung tumor rates were detected (Orthoefer et al., 1981; Kaplan et al., 1982, 1983; White et al., 1983). The only study in which lung tumor incidences were increased in animals exposed to filtered exhaust was reported by Heinrich et al. (1986a) and Stöber (1986) using NMRI mice.

Attempts to induce significant increases in lung tumors in Syrian hamsters were unsuccessful after inhalation (Heinrich et al., 1982; Heinrich et al., 1986a; Heinrich et al., 1989b; Brightwell et al., 1986) or intratracheal instillation (Kunitake et al., 1986; Ishinishi et al., 1988a). Neither cats (Pepelko and Peirano, 1983 [see Chapter 4]), nor monkeys (Lewis et al., 1986) developed tumors following at 2-year exposure to diesel exhaust. The duration of these exposures, however, may well have been inadequate in these two longer-lived species. Exposure levels were also below the MTD in the monkey studies, and borderline for detection of lung tumor increases in rats.

Kawabata et al. (1986) demonstrated the induction of lung tumors in Fischer 344 rats following intratracheal instillation of diesel particulate matter. Grimmer et al. (1987) showed, not only that an extract of diesel particles was carcinogenic when instilled in the lungs of rats but also that most of the carcinogenicity resided in the portion containing PAHs with four to seven rings.

Alternative exposure routes including dermal exposure and sc injection in mice provided additional evidence for tumorigenic effects of diesel exhaust. Particle extracts applied dermally to mice have been shown to induce significant skin tumor increases in two studies (Kotin et al., 1955; Nesnow et al., 1982). Kunitake et al. (1986) also reported a marginally significant increase in skin papillomas in ICR mice treated with an organic extract from an HD diesel engine. Negative results were reported by Depass et al. (1982) for skin-painting studies using mice and acetone extracts of diesel exhaust particle suspensions. However, in this study the exhaust particles were collected at temperatures of 100 °C,

a temperature that would minimize the condensation of vapor-phase organics and, therefore, reduce the availability of potentially carcinogenic compounds that might normally be present on diesel exhaust particles. A significant increase in the incidence of sarcomas in female C57Bl mice was reported by Kunitake et al. (1986) following sc administration of LD diesel exhaust particle extract at doses of 500 mg/kg. Takemoto et al. (1988) provided additional data for this study and reported an increased tumor incidence in the mice following injection of LD engine exhaust extract at doses of 100 and 500 mg/kg. Results of ip injection of diesel exhaust particulate matter or particle extracts in strain A mice were generally negative (Orthoefer et al., 1981; Pepelko and Peirano, 1983), suggesting that the strain A mouse may not be a good model for testing of diesel emissions.

Experiments using tumor initiators such as DEN, B[*a*]P, DPN, or DBA (Brightwell et al., 1986; Heinrich et al., 1986a; Takemoto et al., 1986) did not provide conclusive results regarding the tumor promoting potential of either filtered or whole diesel exhaust. A report by Heinrich et al. (1982), however, did indicate that filtered exhaust may promote the tumor-initiating effects of DEN in hamsters.

Several reports (Wong et al., 1986; Bond et al., 1990) affirm observations of the potential carcinogenicity of diesel exhaust by providing evidence for DNA damage in rats. These findings are discussed in more detail in Chapter 9. Evidence for the mutagenicity of organic agents present in diesel engine emissions is also provided in Chapter 8.

It appears reasonably certain that with adequate exposures, inhalation of diesel exhaust will induce lung cancer in rats and in at least some strains of mice. The relationship between exposure levels and response, however, is less clearcut. Although significant increases in lung tumors were not reported at concentrations less than about 2 $mg/m^3$, the response at higher concentrations varies considerably. A significant percentage of this variation can probably be attributed to the exposure regime. A better method than concentration alone for assessing exposure-response relationships could be achieved by comparing cumulative exposure (concentration $\times$ daily exposure duration $\times$ days of exposure). Only those studies conducted for a sufficient length of time ($\geq$24 mo) for expression of carcinogenic responses have been included in this analysis. Examination of the rat data, shown in Table 7-5 and plotted in Figure 7-1 reveals that most studies indicate a trend of increasing tumor incidence at exposures exceeding $1 \times 10^4$ $mg \cdot h/m^3$.

## TABLE 7-5. CUMULATIVE (CONCENTRATION × TIME) EXPOSURE DATA FOR RATS EXPOSED TO WHOLE DIESEL EXHAUST

| Study | Exposure Rate/Duration (h/week, mo) | Total Exposure Time (h) | Particle Concentration ($mg/m^3$) | Cumulative Exposure ($mg \cdot h/m^3$) Per Week | Total | Tumor Incidence (%)[a] |
|---|---|---|---|---|---|---|
| Mauderly et al. (1987) | 35, 30 | 4,200 | 0 | 0 | 0 | 0.9 |
| | 35, 30 | 4,200 | 0.35 | 12.25 | 1,470 | 1.3 |
| | 35, 30 | 4,200 | 3.5 | 122.5 | 14,700 | 3.6 |
| | 35, 30 | 4,200 | 7.1 | 248.5 | 29,820 | 12.8 |
| Heinrich et al. (1986b) | 95, 35 | 13,300 | 0 | 0 | 0 | 0 |
| | 95, 35 | 13,300 | 4.24 | 402.8 | 56,392 | 17.8 |
| Ishinishi et al. (1988b) | 96, 30 | 11,520 | 0 | 0 | 0 | 3.3 |
| (Light-duty engine) | 96, 30 | 11,520 | 0.1 | 9.6 | 1,152 | 2.4 |
| | 96, 30 | 11,520 | 0.4 | 38.4 | 4,608 | 0.8 |
| | 96, 30 | 11,520 | 1.1 | 105.6 | 12,672 | 4.1 |
| | 96, 30 | 11,520 | 2.3 | 220.8 | 26,496 | 2.4 |
| (Heavy-duty engine) | 96, 30 | 11,520 | 0 | 0 | 0 | 0.8 |
| | 96, 30 | 11,520 | 0.5 | 48.0 | 5,760 | 0.8 |
| | 96, 30 | 11,520 | 1.0 | 96.0 | 11,520 | 0 |
| | 96, 30 | 11,520 | 1.8 | 172.8 | 20,736 | 3.3 |
| | 96, 30 | 11,520 | 3.7 | 355.2 | 42,624 | 6.5 |
| Brightwell et al. (1989) | 80, 24 | 7,680 | 0 | 0 | 0 | 1.2 |
| | 80, 24 | 7,680 | 0.7 | 56.0 | 5,376 | 0.7 |
| | 80, 24 | 7,680 | 2.2 | 176.0 | 16,896 | 9.7 |
| | 80, 24 | 7,680 | 6.6 | 528.0 | 50,688 | 38.5 |

**TABLE 7-5 (cont'd). CUMULATIVE (CONCENTRATION × TIME) EXPOSURE DATA FOR RATS EXPOSED TO WHOLE DIESEL EXHAUST**

| Study | Exposure Rate/Duration (h/week, mo) | Total Exposure Time (h) | Particle Concentration ($mg/m^3$) | Cumulative Exposure ($mg \cdot h/m^3$) Per Week | Total | Tumor Incidence (%)[a] |
|---|---|---|---|---|---|---|
| Kaplan et al. (1983) | 140, 15 | 8,400 | 0 | 0 | 0 | 0 |
| | 140, 15 | 8,400 | 0.25 | 35 | 2,100 | 3.3 |
| | 140, 15 | 8,400 | 0.75 | 105 | 6,300 | 10.0 |
| | 140, 15 | 8,400 | 1.5 | 210 | 12,600 | 3.3 |
| Iwai et al. (1986) | 56, 24 | 5,376 | 0 | 0 | 0 | 0 |
| | 56, 24 | 5,376 | 4.9 | 274.4 | 26,342 | 36.8 |
| Takemoto et al. (1986) | 16, 18-24 | 1,152-1,536 | 0 | 0 | 0 | 0 |
| | 16, 18-24 | 1,152-1,536 | 2-4 | 32-64 | 3,456-4,608 | 0 |
| Karagianes et al. (1981) | 30, 20 | 2,400 | 0 | 0 | 0 | 0 |
| | 30, 20 | 2,400 | 8.3 | 249 | 19,920 | 16.6 |

[a]Combined data for males and females.



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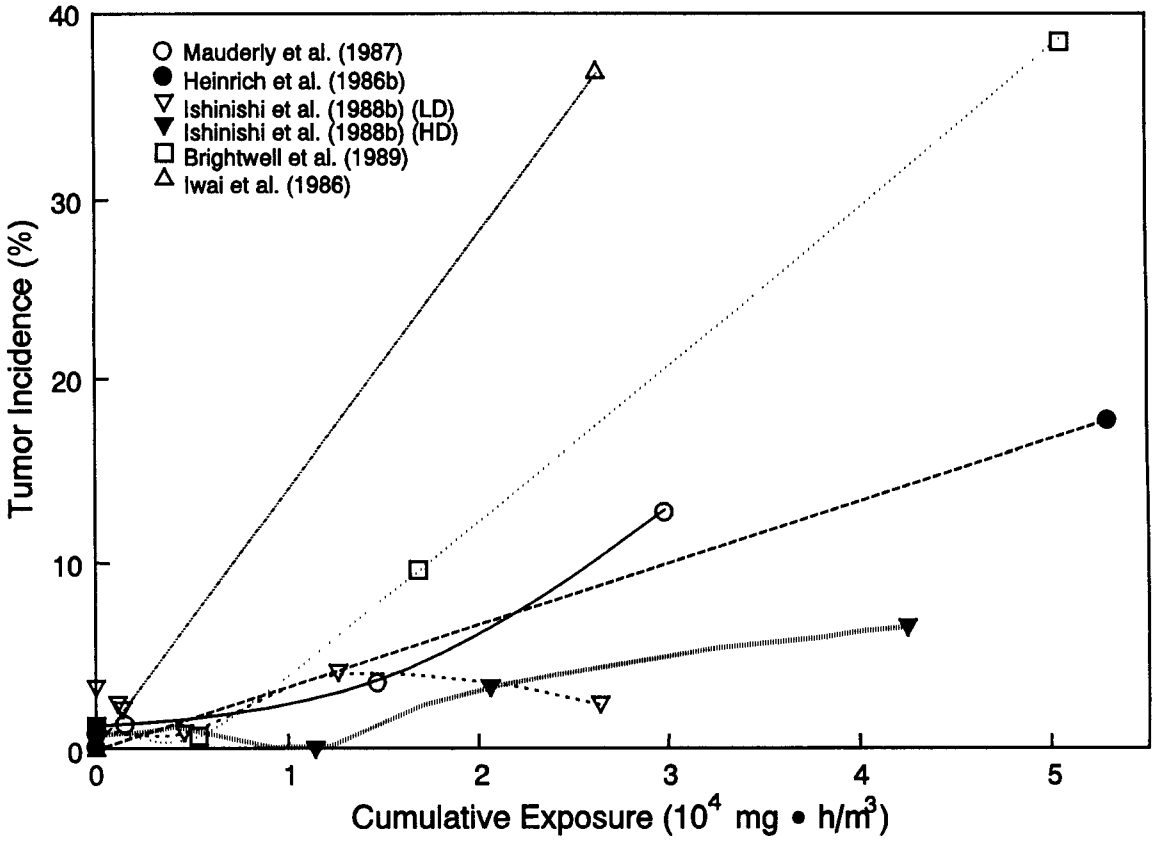


**Figure 7-1. Cumulative exposure data for rats exposed to whole diesel exhaust.**

A similar comparison could not be adequately made for mice, because experimental designs were not comparable. The study reported by Stöber (1986), for example, involved lifetime exposure following weaning. In the studies reported by Pepelko and Peirano (1983), however, Sencar mice were exposed from conception, when they are presumably more sensitive to tumor induction, and sacrificed at 15 mo, whereas strain A mice were sacrificed at 9 mo of age because of the rapid increase in the incidence of lung tumors in controls.

Although the preceding analysis does account for total inspired dose, it does not account for delayed particle clearance at higher exposure levels. The analysis might be improved further by comparing tumor response with lung burden of particulate matter. This analysis could account for not only differences in exposure times but also for overload inhibition of clearance at high exposure levels. Although such data was only reported in the Mauderly et al. (1987) study, it could be estimated in the other studies by using available estimates of respiration along with deposition and clearance models. The extrapolation

models (Appendix A) and the qualitative/quantitative evaluations of Chapter 11 attempt this relative to human exposure.

To evaluate accurately the carcinogenic risk to humans from diesel engine emissions it is important to ascertain the fraction or fractions of exhaust responsible for induction of lung tumors. Several of the previously discussed studies indicated that only whole (unfiltered) diesel exhaust is tumorigenic or carcinogenic and that these properties are eliminated or greatly minimized in filtered diesel exhaust exposure. In one study (Stöber, 1986), however, a significant increase in lung tumors was seen in mice exposed to filtered exhaust. Heinrich et al. (1982) also provided some evidence suggesting that the gaseous fraction promoted the tumorigenic effects of DEN. Nevertheless, because of the lack of positive data in rats and the limited positive data in mice, the tumorigenicity of the gaseous fraction must be considered to be unresolved.

The relative contribution of the carbon core of the diesel particles versus organics adsorbed to the surface of the particles to cancer induction is still somewhat uncertain. The primary evidence for the importance of the adsorbed organics is the presence of known carcinogens among these chemicals. These include polycyclic aromatics as well as nitroaromatics as described in Chapters 2 and 3. Organic extracts of particles have also been shown to induce tumors in a variety of injection, intratracheal instillation and skin painting studies, and Grimmer et al. (1987) has, in fact, shown that the great majority of the carcinogenic potential following intratracheal instillation resided in the fraction containing four- to seven-ring PAHs.

Evidence for the importance of the carbon core is provided by studies of Kawabata et al. (1986), that showed induction of lung tumors following intratracheal instillation of CB that contained no more than traces of organics and studies of Heinrich (1990) that indicated that exposure via inhalation to CB(Printex 90) particles induced lung tumors at concentrations similar to those effective in diesel studies. Other particles of low solubility such as titanium dioxide (Lee et al., 1986) have also been shown to induce lung tumors, although at much higher concentrations than necessary for carbon particles or diesel exhaust. Pyrolyzed pitch, on the other hand, essentially lacking a carbon core but having PAH concentrations at least three orders of magnitude greater than diesel exhaust, was no more effective in tumor induction than was diesel exhaust (Heinrich et al., 1986b). These studies

suggest that the insoluble carbon core of the particle is at least as important as the organic components and possibly more so for lung tumor induction at high particle concentrations ($>2$ mg/m$^3$). A more detailed discussion of this issue can be found in Chapter 11.

In summary, based on positive inhalation exposure data in rats and mice, intratracheal instillation in rats, and injection or skin painting in mice and supported by positive mutagenicity studies, the evidence for carcinogenicity of diesel exhaust is considered to be adequate. The contribution of the various fractions of diesel exhaust to the carcinogenic response is less certain. The effects of the gaseous phase are equivocal. The presence of known carcinogens adsorbed to diesel particles and the demonstrated tumorigenicity of particle extracts in a variety of injection, instillation- and skin-painting studies provides evidence for the involvement of the organic fraction. Studies showing that pure carbon particles can also induce tumors, on the other hand, indicate that the carbon core of the diesel particle is also involved in the carcinogenic process.

A summary of studies assessing the tumorigenic and carcinogenic effects in laboratory animals following inhalation exposure to diesel exhaust is presented in Table 7-1.

# REFERENCES


Bond, J. A.; Johnson, N. F.; Snipes, M. B.; Mauderly, J. L. (1990) DNA adduct formation in rat alveolar type II cells: cells potentially at risk for inhaled diesel exhaust. Environ. Mol. Mutagen. 16: 64-69.

Brightwell, J.; Fouillet, X.; Cassano-Zoppi, A.-L.; Gatz, R.; Duchosal, F. (1986) Neoplastic and functional changes in rodents after chronic inhalation of engine exhaust emissions. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 471-485. (Developments in toxicology and environmental science: v. 13).

Brightwell, J.; Fouillet, X.; Cassano-Zoppi, A.-L.; Bernstein, D.; Crawley, F.; Duchosal, R.; Gatz, R.; Perczel, S.; Pfeifer, H. (1989) Tumors of the respiratory tract in rats and hamsters following chronic inhalation of engine exhaust emissions. J. Appl. Toxicol. 9: 23-31.

Carpenter, K.; Johnson, J. H. (1980) Analysis of the physical characteristics of diesel particulate matter using transmission electron microscope techniques. SAE Trans. 88: 2743-2759.

Cuddihy, R. G.; Griffith, W. C.; McClellan, R. O. (1984) Health risks from light-duty diesel vehicles. Environ. Sci. Technol. 18: 14A-21A.

Depass, L. R.; Chen, K. C.; Peterson, L. G. (1982) Dermal carcinogenesis bioassays of diesel particulates and dichloromethane extract of diesel particulates in C3H mice. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency 1981 diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 321-326. (Developments in toxicology and environmental science: v. 10).

Grimmer, G.; Brune, H.; Deutsch-Wenzel, R.; Dettbarn, G.; Jacob, J.; Naujack, K.-W.; 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. (Shannon, Irel.) 37: 173-180.

Heinrich, U. (1990) Results of long-term inhalation exposure of rats to carbon black "Printex 90" [letter to Dr. Lester D. Grant]. Presented at: U.S. Environmental Protection Agency peer review workshop on the Health Assessment Document for Diesel Emissions; July; Research Triangle Park, NC.

Heinrich, U.; Peters, L.; Funcke, W.; Pott, F.; Mohr, U.; Stöber, W. (1982) Investigation of toxic and carcinogenic effects of diesel exhaust in long-term inhalation exposure of rodents. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 225-242. (Developments in toxicology and environmental science: v. 10).

Heinrich, U.; Muhle, H.; Takenaka, S.; Ernst, H.; Fuhst, R.; Mohr, U.; Pott, F.; Stöber, W. (1986a) Chronic effects on the respiratory tract of hamsters, mice, and rats after long-term inhalation of high concentrations of filtered and unfiltered diesel engine emissions. J. Appl. Toxicol. 6: 383-395.

Heinrich, U.; Pott, F.; Rittinghausen, S. (1986b) Comparison of chronic inhalation effects in rodents after long-term exposure to either coal oven flue gas mixed with pyrolized pitch or diesel engine exhaust. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite syposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 441-457. (Developments in toxicology and environmental science: v. 13).

Heinrich, U.; Peters, L.; Fuhst, R.; Mohr, U. (1989a) The effect of automotive exhaust exposure on the carcinogenicity of dipentylnitrosamine (DPN) in the respiratory tract of rats. Exp. Pathol. 37: 51-55.

Heinrich, U.; Mohr, U.; Fuhst, R.; Brockmeyer, C. (1989b) Investigation of a potential cotumorigenic effect of the dioxides of nitrogen and sulfur, and of diesel-engine exhaust, on the respiratory tract of Syrian golden hamsters. Cambridge, MA: Health Effects Institute; research report no. 26. Available from: NTIS, Springfield, VA; PB90-111147.

Huisingh, J.; Bradow, R.; Jungers, R.; Claxton, L.; Zweidinger, R.; Tejada, S.; Bumgarner, J.; Duffield, F.; Waters, M.; Simmon, V. F.; Hare, C.; Rodriguez, C.; Snow, L. (1978) Application of bioassay to the characterization of diesel particle emissions. In: Waters, M. D.; Nesnow, S.; Huisingh, J. L.; Sandhu, S. S.; Claxton, L., eds. Application of short-term bioassays in the fractionation and analysis of complex environmental mixtures: [proceedings of a symposium; February; Williamsburg, VA]. New York, NY: Plenum Press; pp. 383-418. (Hollaender, A.; Probstein, F.; Welch, B. L., eds. Environmental science research: v. 15).

International Agency for Research on Cancer. (1989) Diesel and gasoline engine exhausts and some nitroarenes. Lyon, France: World Health Organization; p. 104. (IARC monographs on the evaluation of carcinogenic risks to humans: v. 46).

Ishinishi, N.; Inamasu, T.; Hisanaga, A.; Tanaka, A.; Hirata, M.; Ohyama, S. (1988a) Intratracheal instillation study of diesel particulate extracts in hamsters. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 209-216.

Ishinishi, N.; Kuwabara, N.; Takaki, Y.; Nagase, S.; Suzuki, T.; Nakajima, T.; Maejima, K.; Kato, A.; Nakamura, M. (1988b) Long-term inhalation experiments on diesel exhaust. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 11-84.

Iwai, K.; Udagawa, T.; Yamagishi, M.; Yamada, H. (1986) Long-term inhalation studies of diesel exhaust on F344 SPF rats. Incidence of lung cancer and lymphoma. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 349-360. (Developments in toxicology and environmental science: v. 13).

Kaplan, H. L.; MacKenzie, W. F.; Springer, K. J.; Schreck, R. M.; Vostal, J. J. (1982) A subchronic study of the effects of exposure of three species of rodents to diesel exhaust. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency diesel emission symposium; October, 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 161-182. (Developments in toxicology and environmental science: v. 10).

Kaplan, H. L.; Springer, K. J.; MacKenzie, W. F. (1983) Studies of potential health effects of long-term exposure to diesel exhaust emissions. San Antonio, TX: Southwest Research Institute; SwRI project no. 01-0750-103.

Karagianes, M. T.; Palmer, R. F.; Busch, R. H. (1981) Effects of inhaled diesel emissions and coal dust in rats. Am. Ind. Hyg. Assoc. J. 42: 382-391.

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. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 213-222. (Developments in toxicologial and environmental science: v. 13).

Kotin, P.; Falk, H. L.; Thomas, M. (1955) Aromatic hydrocarbons: III. presence in the particulate phase of diesel-engine exhausts and the carcinogenicity of exhaust extracts. AMA Arch. Ind. Health 11: 113-120.

Kunitake, E.; Shimamura, K.; Katayama, H.; Takemoto, K.; Yamamoto, A.; Hisanaga, A.; Ohyama, S.; Ishinishi, N. (1986) Studies concerning carcinogenesis of diesel particulate extracts following intratracheal instillation, subcutaneous injection, or skin application. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 235-252. (Developments in toxicology and environmental science: v. 13).

Kunitake, E.; Imase, A.; Shimamura, K.; Ishinishi, N.; Hisanaga, A.; Tanaka, A. (1988) Skin application and subcutaneous injection experiments of diesel particulate extracts using ICR mice and nude mice. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 217-225.

Lee, K. P.; Henry, N. W., III; Trochimowicz, H. J.; Reinhardt, C. F. (1986) Pulmonary response to impaired lung clearance in rats following excessive $TiO_2$ dust deposition. Environ. Res. 41: 144-167.

Lewis, T. R.; Green, F. H. Y.; Moorman, W. J.; Burg, J. A. R.; Lynch, D. W. (1986) A chronic inhalation toxicity study of diesel engine emissions and coal dust, alone and combined. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 361-380. (Developments in toxicology and environmental science: v. 13).

Mauderly, J. L.; Jones, R. K.; Griffith, W. C.; Henderson, R. F.; McClellan, R. O. (1987) Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam. Appl. Toxicol. 9: 208-221.

Mauderly, J. L.; Snipes, M. B.; Barr, E. B.; Bechtold, W. E.; Henderson, R. F.; Mitchell, C. E.; Nikula, K. J.; Thomassen, D. G. (1991) Influence of particle-associated organic compounds on carcinogenicity of diesel exhaust. Presented at: eighth Health Effects Institute annual conference; April; Colorado Springs, CO. Cambridge, MA: Health Effects Institute.

Mohr, U.; Takenaka, S.; Dungworth, D. L. (1986) Morphologic effects of inhaled diesel engine exhaust on lungs of rats: comparison with effects of coal oven flue gas mixed with pyrolized pitch. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 459-470. (Developments in toxicology and environmental science: v. 13).

Mokler, B. V.; Archibeque, F. A.; Beethe, R. L.; Kelly, C. P. J.; Lopez, J. A.; Mauderly, J. L.; Stafford, D. L. (1984) Diesel exhaust exposure system for animal studies. Fundam. Appl. Toxicol. 4: 270-277.

Nesnow, S.; Evans, C.; Stead, A.; Creason, J.; Slaga, T. J.; Triplett, L. L. (1982) Skin carcinogenesis studies of emission extracts. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 295-320. (Developments in toxicology and environmental science: v. 10).

Nikula, K. J.; Snipes, M. B.; Barr, E. B.; Mauderly, J. L. (1991) Histopathology and lung tumor responses in rats exposed to diesel exhaust or carbon black. In: Annual report of the Inhalation Toxicology Research Institute operated for the United States Department of Energy by the Lovelace Biomedical and Environmental Research Institute: October 1, 1990 though September 30, 1991. Albuquerque, NM: Inhalation Toxicology Research Institute, Lovelace biomedical and Environmental Research Institute; pp. 87-129; report no. LMF-134.

Nikula, K. J.; Snipes, M. B.; Barr, E. B.; Griffith, W. C.; Henderson, R. F.; Mauderly, J. L. (1994) Influence of particle-associated organic compounds on the carcinogenicity of diesel exhaust. In: Mohr, U.; Dungworth, D. L.; Mauderly, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 565-568.

Orthoefer, J. G.; Moore, W.; Kraemer, D. (1981) Carcinogenicity of diesel exhaust as tested in strain A mice. Environ. Int. 5: 461-471.

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

Shefner, A. M.; Collins, B. R.; Dooley, L.; Fiks, A.; Graf, J. L.; Preache, M. M. (1982) Respiratory carcinogenicity of diesel fuel emissions interim results. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency 1981 diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 329-350. (Developments in toxicology and environmental science: v. 10).

Stöber, W. (1986) Experimental induction of tumors in hamsters, mice and rats after long-term inhalation of filtered and unfiltered diesel engine exhaust. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 421-439. (Developments in toxicology and environmental science: v. 13).

Takaki, Y.; Kitamura, S.; Kuwabara, N.; Fukuda, Y. (1989) Long-term inhalation studies of exhaust from diesel engine in F-344 rats: the quantitative relationship between pulmonary hyperplasia and anthracosis. Exp. Pathol. 37: 56-61.

Takemoto, K.; Yoshimura, H.; Katayama, H. (1986) Effects of chronic inhalation exposure to diesel exhaust on the development of lung tumors in di-isopropanol-nitrosamine-treated F344 rats and newborn C57BL and ICR mice. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 311-327. (Developments in toxicology and environmental science: v. 13).

Takemoto, K.; Katayama, H.; Kuwabara, T.; Hasumi, M. (1988) Carcinogenicity by subcutaneous administration of diesel particulate extracts in mice. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 227-234.

Vuk, C. T.; Jones, M. A.; Johnson, J. H. (1976) The measurement and analysis of the physical character of diesel particulate emissions. SAE Trans. 85: 556.

White, H.; Vostal, J. J.; Kaplan, H. L.; MacKenzie, W. F. (1983) A long-term inhalation study evaluates the pulmonary effects of diesel emissions [letter]. J. Appl. Toxicol. 3: 332.

Wong, D.; Mitchell, C. E.; Wolff, R. K.; Mauderly, J. L.; Jeffrey, A. M. (1986) Identification of DNA damage as a result of exposure of rats to diesel engine exhaust. Carcinogenesis (London) 7: 1595-1597.

Zamora, P. O.; Gregory, R. E.; Brooks, A. L. (1983) *In vitro* evaluation of the tumor-promoting potential of diesel-exhaust-particle extracts. J. Toxicol. Environ. Health 11: 187-197.

# 8. EPIDEMIOLOGIC STUDIES OF THE CARCINOGENICITY OF EXPOSURE TO DIESEL EMISSIONS

## 8.1 INTRODUCTION

Emissions from diesel engine exhaust are made up of toxicants that include oxides of nitrogen and sulfur, carbon monoxide, and particulate matter consisting of a carbon core with many organic compounds, especially the polycyclic aromatic hydrocarbons adsorbed on the surface. Diesel engine exhaust contains about 100 times more particulate matter than gasoline engine exhaust.

In this chapter, various mortality and morbidity studies of the health effects of exposure to diesel engine emissions are reviewed. Although an attempt was made to cover all the relevant studies, a number of studies are not included for several reasons. First, the change from steam to diesel engines in locomotives began in 1935 and was about 95% complete by 1959 (Garshick et al., 1988). Diesel buses also were introduced about the same time. Therefore, exposure to diesel exhaust was less common, and the follow-up period for studies conducted prior to 1959 (Raffle, 1957; Kaplan, 1959) was not long enough to cover the long latency period of lung cancer. The usefulness of the studies in evaluating the carcinogenicity of diesel exhaust is greatly reduced; thus, these studies are not considered here.

Second, hypothesis-generating studies were excluded from this review because the findings of such studies need subsequent confirmation by definitive studies (Silverman et al., 1983; Schenker et al., 1984; Buiatti et al., 1985; Flodin et al., 1987; Siemiatycki et al., 1988).

Third, studies in which exposure to diesel exhaust was uncertain or was defined as motor exhaust (which includes both gasoline and diesel exhaust) were excluded from the review as they would have contributed little to the evaluation of the carcinogenicity of diesel exhaust (Waxweiler et al., 1973; Ahlberg et al., 1981; Stern et al., 1981; Vineis and Magnani, 1985; Gustafsson et al., 1986; Silverman et al., 1986; Jensen et al., 1987; Garland et al., 1988; Risch et al., 1988).

Fourth, the study by Coggon et al. (1984) was not included because the occupational information abstracted from death certificates had not been validated; this would have resulted in limited information.

Three types of studies of the health effects of exposure to diesel engine emissions are reviewed in this chapter: (1) cohort studies, (2) case-control studies of lung cancer, and (3) case-control studies of bladder cancer. In the cohort studies, the cohorts of heavy construction equipment operators, railroad and locomotive workers, and bus garage employees were studied retrospectively to determine increased mortality and morbidity resulting from exposures to varying levels of diesel emissions in the workplace. A total of seven cohort mortality, eight lung cancer case-control, and seven bladder cancer case-control studies are considered in this section.

## 8.2 COHORT STUDIES

### 8.2.1 Waller (1981): Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes

A retrospective mortality study of a cohort of London transport workers was conducted to determine if there was an excess of deaths from lung cancer that could be attributed to diesel exhaust exposure. Nearly 20,000 male employees aged 45 to 64 were followed for the 25-year period between 1950 and 1974, constituting a total of 420,700 man-years at risk. These were distributed among five job categories: drivers, garage engineers, conductors, motormen or guards, and engineers (works). Most employees lived in the greater London area. Lung cancer cases occurring in this cohort were ascertained only from death certificates of individuals who died while still employed, or if retired, following diagnosis. Expected death rates were calculated by applying greater London death rates to the population at risk within each job category. Data were calculated in 5-year periods and 5-year age ranges, finally combining the results to obtain the total expected deaths in the required age range of 45 to 64 for the calendar period from 1950 to 1974. A total of 667 cases of lung cancer was reported, compared with 849 expected, to give a mortality ratio of 79%. In each of the five job categories, the observed numbers were below those expected. Engineers in garages had the highest mortality ratio (90%) but this did not differ

significantly from the other job categories. Environmental sampling was done at one garage, on 1 day in 1979, for benzo[*a*]pyrene concentrations and was compared with corresponding values recorded in 1957. Concentrations of benzo[*a*]pyrene recorded in 1957 were at least 10 times greater than those measured in 1979.

This study has several methodologic limitations. The lung cancer deaths ascertained for the study were those that occurred while the worker was employed (the worker either died of lung cancer or retired after lung cancer was diagnosed). Although man-years at risk were based on the entire cohort, no attempt was made to trace or evaluate the individuals who had resigned from the London transport company for any other reason. Hence, the information on resignees who may have had significant exposure to diesel exhaust, and lung cancer deaths among them, was not available for analysis. This fact may have lead to a dilution effect, resulting in underascertainment of observed lung cancer deaths and underestimation of mortality ratios. Eligibility criteria for inclusion in the cohort, such as starting date and length of service with the company, were not specified. Because an external comparison group was used to obtain expected number of deaths, the resulting mortality ratios were less than one; this may be a reflection of the "healthy worker effect". Investigators also did not categorize the five job categories by levels of diesel exhaust exposure nor did they use an internal comparison group to derive risk estimates.

The age range considered for this study was limited (45 to 64 years of age) for the period between 1950 and 1964. It is not clear whether this age range was applied to calendar year 1950 or 1964 or at the mid-point of this 25-year follow-up period. No analyses were presented either by latency or by duration of employment (surrogate for exposure). The environmental survey based on benzo[*a*]pyrene concentrations suggests that the cohort in their earlier years was exposed to much higher concentrations of environmental contaminants than current concentrations. It is not clear when the reduction in benzo[*a*]pyrene concentration occurred since there are no environmental readings available between 1957 and 1979. It is also important to note that the concentrations of benzo[*a*]pyrene inside the garage were not very different from those outside the garage in 1957, thus indicating that the exposure for garage workers was not much different than for the general population. Lastly, no data were collected on smoking habits.

### 8.2.2 Howe et al. (1983): Cancer Mortality (1965 to 1977) in Relation to Diesel Fume and Coal Exposure in a Cohort of Retired Railroad Workers

This is a retrospective cohort study of the mortality experience of 43,826 male pensioners of the Canadian National Railroad (CNR) between 1965 and 1977. Members of this cohort consisted of male CNR pensioners who had retired before 1965 and who were known to be alive at the start of that year, as well as those who retired between 1965 and 1977. The records were obtained from a computer file that is regularly updated and used by the company for payment of pensions. To receive a pension, each pensioner must provide, on a yearly basis, evidence to the effect that he is alive. Specific cause of death among members of this cohort was ascertained by linking these records to the Canadian Mortality Data Base, which contains records of all deaths registered in Canada since 1950. Of the 17,838 deaths among members of the cohort between 1965 and 1977, 16,812 (94.4%) were successfully linked to a record on the mortality file. A random sample manual check on unlinked data revealed that failure to link was mainly due to some missing information on the death records.

Occupation at time of retirement was used by the Department of Industrial Relations to classify workers into three diesel fume and coal dust exposure categories: (1) nonexposed, (2) possibly exposed, and (3) probably exposed. Person-years of observation were calculated and classified by age at observation in 5-year age groups (35 to 39, 40 to 44, . . ., 80 to 84, and $\geq$85 years). The observed deaths were classified by age at death for different cancers, for all cancers combined, and for all causes of death combined. Standard mortality ratios (SMRs) were then calculated using rates of the Canadian population for the period between 1965 and 1977.

Both total mortality (SMR = 95, $p < 0.001$) and all cancer deaths (SMR = 99, $p > 0.05$) were close to that expected for the entire cohort. Analysis by exposure to diesel fume levels in the three categories (nonexposed, possibly exposed, and probably exposed) revealed an increased relative risk for lung cancer among workers with increasing exposure to diesel fumes. The relative risk for nonexposed workers was presumed to be 1.0; for those possibly exposed, the relative risk was elevated to 1.2, which was statistically significant ($p = 0.013$); and, for those probably exposed, it was elevated to 1.35, which was statistically highly significant ($p = 0.001$). The corresponding rates for exposure to varying

levels of coal dust were very similar at 1.00, 1.21 ($p = 0.012$), and 1.35 ($p = 0.001$), respectively. The trend tests were highly significant for both exposures ($p < 0.001$). Analysis performed after the exclusion of individuals who worked in the maintenance of steam engines, hence exposed to high levels of asbestos, yielded the risk of lung cancer to be 1.00, 1.21, and 1.33 for the nonexposed, possibly exposed, and probably exposed to diesel exhaust, respectively, with a highly significant trend ($p < 0.001$).

An analysis done on individuals who retired prior to 1950 showed the relative risk of lung cancer among nonexposed, possibly exposed, and probably exposed to be 1.00, 0.70, and 0.44, respectively, based on less than 15 deaths in each category. A similar analysis of individuals who retired after 1950 found the results in the same categories to be 1.00, 1.23, and 1.40, respectively. Although retirement prior to 1950 indicated exposure to coal dust alone, retirement after 1950 shows the results of mixed exposure to coal dust and diesel fumes. As there was considerable overlap between occupations involving probable exposure to diesel fumes and probable exposure to coal dust and as most members of the cohort were employed during the years in which the transition from coal to diesel occurred, it was difficult to distinguish whether lung cancer was associated with exposure to coal dust or diesel fumes or a mixture of both.

Although this study showed a highly significant dose-response relationship between diesel fumes and lung cancer, it has some methodological limitations. There were concurrent exposures to both diesel fumes and coal dust during the transition period; therefore, misclassification of exposure may have occurred, because only occupation at retirement was available for analysis. It is possible that the elevated response observed for lung cancer was due to the combined effects of exposure to both coal dust and diesel fumes and not just one or the other. However, it should be noted that so far coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners. No information was provided on duration of employment in either diesel work or the coal dust-related jobs for other than those jobs held at retirement. Therefore, it was not possible to evaluate whether this omission would have led to an under- or overestimate of the true relative risk. Furthermore, a lack of information on potential confounders such as smoking makes the interpretation of the excess risk of lung cancer even more difficult. Information on cause of death was

acquired from the mortality data linkage. There is a possibility that the cause of death may have been misclassified because of miscoding of the underlying cause of death.

### 8.2.3 Rushton et al. (1983): Epidemiological Survey of Maintenance Workers in the London Transport Executive Bus Garages and Chiswick Works

This is a retrospective mortality cohort study of male maintenance workers employed for at least 1 continuous year between January 1, 1967, and December 31, 1975, at 71 London transport bus garages (also known as rolling stock) and at Chiswick Works. For all men, the following information was obtained from computer listings: surname with initials, date of birth, date of joining company, last or present jobs, and location of work. For those individuals who left their job, date of and reason for leaving were also obtained. For those who died in service or after retirement and for men who had resigned, full name and last known address were obtained from an alphabetical card index in the personnel department. Additional tracing of individuals who had left was carried out through social security records. The area of their residence was assumed to be close to their work; therefore their place of work was coded as their residence. There were 100 different job titles that were coded into 20 broader groups. These 20 groups were not ranked for diesel exhaust exposure though. The reason for leaving was coded as died in service, retired, or other. The underlying cause of death was coded using the eighth revision of the International Classification of Diseases (ICD). Person-years were calculated from date of birth and dates of entry to and exit from the study using the man-years computer language program. These were then subdivided into 5-year age and calendar period groups. The expected number of deaths was calculated by applying the 5-year age and calendar period death rates of the comparison population to the person-years of corresponding groups. The mortality experience of the male population in England and Wales was used as the comparison population. Significance values were calculated for the difference between the observed and expected deaths, assuming a Poisson distribution.

The number of person-years of observation totaled 50,008 and was contributed by 8,490 individuals in the study with a mean follow-up of 5.9 years. Only 2.2% (194) of the men were not traced. Observed deaths from all causes were significantly lower than expected (observed = 495, $p < 0.001$). The observed deaths from all neoplasms and cancer

of the lung were approximately the same as those expected. The only significant excess observed for cancer of the liver and gall bladder at Chiswick Works was based on four deaths ($p < 0.05$). A few job groups showed a significant excess of risks for various cancers. Cancer of the lung was elevated in the general hand category (SMR = 133, observed = 48, $p < 0.03$). Cancer of the liver and gall bladder (observed = 2, $p < 0.01$) and cancer of the bladder (observed = 3, $p < 0.01$) were significant in the job categories of inspector and progress hand, respectively. Welders showed an excess of lung cancer (observed = 3, $p < 0.05$), bus mechanics had an excess of cancer of the brain (observed = 4, $p < 0.04$), and painters had an excess of bladder cancer (observed = 2, $p < 0.02$). All the excess deaths observed for the various job groups, except for the general hand category, were based on very small numbers (usually smaller than five) and merited cautious interpretation. Although the lung cancer excess in the general hand category was based on 48 cases (SMR = 133), given the fact that there was no adjustment for confounding variables such as smoking, the results should be interpreted cautiously.

This mortality study of London transport maintenance workers did not demonstrate any cancer excesses based on a large number of cases; this needs further exploration. Its limitations include the small sample size, short duration of follow-up (average of only 6 years), and lack of sufficient latency period, make this study inadequate to draw any conclusions. The number of deaths by different causes and among the various job groups was too small to allow any meaningful conclusions. Details of work history were not obtained to permit any analysis by diesel exhaust exposure. Death information was ascertained from death certificates with inherent problems of inaccuracy, misdiagnosis, and errors in coding, and it was not known whether a trained nosologist coded the death certificates. No adjustments were made for the confounding effects of smoking and socioeconomic factors.

### 8.2.4 Wong et al. (1985): Mortality Among Members of a Heavy Construction Operators Union with Potential Exposure to Diesel Exhaust Emissions

This is a retrospective mortality study conducted on a cohort of 34,156 male members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. Study cohort members were identified from records maintained at Operating

Engineers' Local Union No. 3-3A in San Francisco, CA. This union has maintained both work and death records on all its members since 1964. Individuals with at least 1 year of membership in this union between January 1, 1964, and December 31, 1978, were included in the study. Work histories of the cohort were obtained from job dispatch computer tapes. The study follow-up period was from January 1964 to December 1978. Death information was obtained from a trust fund, which provided information on retirement dates, vital status, and date of death for those who were entitled to retirement and death benefits. Approximately 50% of the cohort had been union members for less than 15 years, whereas the other 50% had been union members for 15 years or more. The average duration of membership was 15 years. As of December 31, 1978, 29,046 (85%) cohort members were alive, 3,345 (9.8%) were dead, and 1,765 (5.2%) remained untraced. Vital status of 10,505 members who had left the union as of December 31, 1978, were ascertained from the Social Security Administration. Death certificates were obtained from appropriate state health departments. Altogether, 3,243 deaths (for whom death certificates were available) in the cohort were coded using the seventh revision of the ICD. Death certificates could not be obtained for 102 individuals, only the date of death was available; these individuals were included in the calculation of the SMR for all causes of death but were deleted from the cause-specific SMR analyses. Expected deaths and SMRs were calculated using the U.S. national age-sex-race cause-specific mortality rates for 5-year time periods between 1964 and 1978. The entire cohort population contributed to 372,525.6 person-years in this 5-year study period.

A total of 3,345 deaths was observed, compared with 4,109 expected. The corresponding SMR for all causes was 81.4 ($p = 0.01$), which confirmed the "healthy worker effect". A total of 817 deaths was attributed to malignant neoplasms, slightly fewer than the 878.34 expected based on U.S. white male cancer mortality rates (SMR = 93.0, $p = 0.05$). Mostly there were SMR deficits for cause-specific cancers, including lung cancer for the entire cohort (SMR = 98.6, observed = 309). The only significant excess SMR was observed for cancer of the liver (SMR = 166.7, observed = 23, $p < 0.05$).

Analysis by length of union membership as a surrogate of duration for potential exposure showed statistically significant increases in SMRs of cancer of the liver (SMR = 424, $p < 0.01$) in the 10- to 14-year membership group and of the stomach

(SMR = 248, $p < 0.05$) in the 5- to 9-year membership group. No cancer excesses were observed in the 15- to 19-year and 20+-year membership groups. Although the SMR for cancer of the lung had a statistically significant deficit in the less than 5-year duration group, it showed a positive trend with increasing length of membership, which leveled off after 10 to 14 years.

Cause-specific mortality analysis by latency period showed a positive trend for SMRs of all causes of death, although all of them were statistically significant deficits, reflecting the diminishing "healthy worker effect". This analysis also demonstrated a statistically significant SMR excess for cancer of the liver (10- to 19-year group, SMR = 257.9). The SMR for cancer of the lung showed a statistically significant deficit for a <10-year latency, but showed a definite positive trend with increasing latency.

In addition to these analyses of the entire cohort, similar analyses were carried out in various subcohorts. Analyses of retirees, 6,678 individuals contributing to 32,670.1 person-years, showed statistically significant increases ($p < 0.01$) in SMRs for all cancers (SMR = 145.3, observed = 389), all causes of death (SMR = 114.5, observed = 1,345), cancer of the digestive system (SMR = 142.4, observed = 103), cancer of the large intestine (SMR = 181.8, observed = 46), cancer of the respiratory system (162.4, observed = 161), cancer of the lung (SMR = 164.1, observed = 155), emphysema (SMR = 277.3, observed = 75), and cirrhosis of the liver (SMR = 173.5, observed = 38). The other two significant excesses ($p < 0.01$) were for lymphosarcoma and reticulosarcoma (SMR = 231.2, observed = 10) and nonmalignant respiratory diseases (SMR = 129.0, observed = 112). Further analysis of the 4,075 retirees (18,677.8 person-years) who retired at age 65 or who retired earlier but had reached the age of 65, revealed statistically significant SMR increases for all cancers (SMR = 114.7, observed = 224, $p = 0.05$), cancer of the lung (SMR = 130, observed = 86, $p < 0.05$), and lymphosarcoma and reticulosarcoma (SMR = 266.5, observed = 8, $p < 0.05$).

To analyze cause-specific mortality by job held (potential exposure to diesel exhaust emissions), 20 functional job titles were used, which were further grouped into three potential categories: (1) high exposure, (2) low exposure, and (3) unknown exposure. A person was classified in a job title if he ever worked on that job. Based on this classification system, if a person had ever worked in a high-exposure job title he was

included in that group, even though he may have worked for a longer time in a low-exposure group or in an unknown exposure group. Information on length of work in any particular job, hence indirect information on potential length of exposure, was not available either.

For the high-exposure group a statistically significant excess was observed for bulldozer operators who had 15 to 19 years of membership and 20+ years of follow-up for cancer of the lung (SMR = 343.4, $p < 0.05$). This excess was based on 5 out of 495 deaths observed in this group of 6,712 individuals who contributed 80,327.6 person-years of observation.

The cause-specific mortality analysis in the low-exposure group revealed statistically significant SMR excesses in individuals who had ever worked as engineers. These excesses were for cancer of the large intestine (SMR = 807.2, observed = 3, $p < 0.05$) among those with 15 to 19 years of membership and length of follow-up of at least 20 years, and cancer of the liver (SMR = 871.9, observed = 3, $p < 0.05$) among those with 10 to 14 years of membership and length of follow-up of 10 to 19 years. There were 7,032 individuals who contributed to 78,402.9 person-years of observation in the low-exposure group.

For the unknown exposure group, a statistically significant SMR was observed for motor vehicle accidents only (SMR = 173.3, observed = 21, $p < 0.05$). There were 3,656 individuals who contributed to 33,388.1 person-years of observation in this category.

No work histories were available for those who started their jobs before 1967 and for those who held the same job prior to and after 1967. This constituted 9,707 individuals (28% of the cohort) contributing to 104,447.5 person-years. Statistically significant SMR excesses were observed for all cancers (SMR = 112, observed = 339, $p < 0.05$) and cancer of the lung (SMR = 119.3, observed = 141, $p < 0.01$). A significant SMR elevation was also observed for cancer of the stomach (SMR = 199.1, observed = 30, $p < 0.01$).

This study demonstrates a statistically significant excess for cancer of the liver, but also shows statistically significant deficits in cancers of the large intestine and rectum. It may be, as the authors suggested, that the liver cancer cases were actually cases resulting from metastases from the large intestine and/or rectum, since tumors of these sites will frequently metastasize to the liver. The excess in liver cancer mortality and the deficits in mortality that are due to cancer of the large intestine and rectum could also, as the authors indicate, be due to misclassification. Both possibilities have been considered by the investigators in their discussion.

Cancer of the lung showed a positive trend with length of membership as well as with latency, although none of the SMRs were statistically significant except for the workers without any work histories. The individuals without any work histories may have been the ones who were in their jobs for the longest period of time, because workers without job histories included those who had the same job before and after 1967 and thus may have worked 12 to 14 years or longer. If they had belonged to the category in which heavy exposure to diesel exhaust emissions was very common for this prolonged time, then the increase in lung cancer, as well as stomach cancer, might be linked to diesel exhaust. Further information on those without work histories should be obtained if possible since such information may be quite informative with regard to the evaluation of the carcinogenicity of diesel exhaust.

The study design is adequate, covers about a 15-year observation period, has a large enough population, and is appropriately analyzed; however, it has too many limitations to permit any conclusions. First, no exposure histories are available. One has to make do with job histories which provide limited information on exposure level. Any person who ever worked at the job or any person working at the same job over any period of time are included in the same category; this would have a dilution effect, since extremely variable exposures were considered in the study. Second, the length of time worked in any particular job is not available. Third, work histories were not available for 9,707 individuals who contributed 104,447.5 person-years, a large proportion of the study cohort (28%). These individuals happen to show the most evidence of a carcinogenic effect. Confounding by alcohol consumption for cancer of the liver and smoking for emphysema and cancer of the lung were not ruled out. Lastly, though 34,156 members were eligible for the study, the vital status of 1,765 individuals was unknown. Nevertheless, they were still considered in the denominator of all the analyses. The investigators fail to mention how the person-year calculation for these individuals was handled. Also, some of the person-years might have been overestimated, as people may have paid the dues for a particular year and then left work. These two causes of overestimation of the denominator may have resulted in some or all the SMRs to be underestimated.

As for the smoking survey, the investigators took a very small sample (133 out of 34,156, which was not even 1%). Of 133, only 107 (80%) participated. It was a systematic

sample, but the authors neglected to mention how the list was prepared for this systematic sample. Hence, the sample may not be representative of the study population, and, with a small sample size, the results are not generalizable. The questionnaire asked only for current smoking history. No detailed history was obtained for the amount smoked or length of smoking history, both of which have a bearing on emphysema as well as lung carcinoma.

### 8.2.5 Edling et al. (1987): Mortality Among Personnel Exposed to Diesel Exhaust

This is a retrospective cohort mortality study of bus company employees, which investigated a possible increased mortality in cardiovascular diseases and cancers from diesel exhaust exposure. The cohort comprised all males employed at five different bus companies in southeastern Sweden between 1950 and 1959. Using information from personnel registers, individuals were classified into one or more categories and could have contributed person-years at risk in more than one exposure category. The study period was from 1951 to 1983; information was collected from the National Death Registry, and copies of death certificates were obtained from the National Bureau of Statistics. Workers who died after age 79 were excluded from the study because diagnostic procedures were likely to be more uncertain at higher ages (according to investigators). The cause-, sex-, and age-specific national death rates in Sweden were applied to the 5-year age categories of person-years of observation to determine expected deaths for all causes, malignant diseases, and cardiovascular diseases. A Poisson distribution was used to calculate p-values and confidence limits for the ratio of observed to expected deaths. The total cohort of 694 men (after loss of 5 men to follow-up) was divided into three exposure categories: (1) clerks with the lowest exposure, (2) bus drivers with moderate exposure, and (3) bus garage workers with highest exposure.

The 694 men provided 20,304 person-years of observation with 195 deaths compared to 237 expected. A deficit in cancer deaths largely accounted for this lower than expected mortality in the total cohort. Among subcohorts, no difference between observed and expected deaths for total mortality, total cancers, or cardiovascular causes was observed for clerks (lowest diesel exposure), bus drivers (moderate diesel exposure), and garage workers

(high diesel exposure). The risk ratios for all three categories were less than one except for cardiovascular diseases among bus drivers, which was 1.1.

When the analysis was restricted to members who had at least a 10-year latency period and either any exposure or an exposure exceeding 10 years, similar results were obtained with fewer neoplasms than expected, whereas cardiovascular diseases showed risk around or slightly above unity.

Five lung cancer deaths were observed among bus drivers who had moderate diesel exhaust exposure while 7.2 were expected. The only other lung cancer death was observed among bus garage workers who had the highest diesel exhaust exposure. The small size of the cohort and poor data on diesel exhaust exposure are among the major limitations of this study. Although lifetime occupational histories were available, no industrial hygiene data were presented to validate the classification of workers into low, moderate, and high exposure to diesel exhaust based on job title. The power of the present study was estimated to be 80% to detect a relative risk of 1.2 for cardiovascular diseases and 1.4 for cancers, but for specific cancer sites, the power was much lower than this. No information was available on confounding effects of smoking and asbestos exposure at the work sites.

### 8.2.6 Boffetta and Stellman (1988): Diesel Exhaust Exposure and Mortality Among Males in the American Cancer Society Prospective Study

Boffetta and Stellman conducted a mortality analysis of 46,981 males whose vital status was known at the end of the first 2 years of follow-up. The analysis was restricted to males aged 40 to 79 years in 1982 who enrolled in the American Cancer Society's prospective mortality study of cancer. Mortality was analyzed in relation to exposure to diesel exhaust exposure and to employment in selected occupations related to diesel exhaust exposure. In 1982, more than 77,000 American Cancer Society volunteers enrolled over 1.2 million men and women from all 50 states, the District of Columbia, and Puerto Rico in a long-term cohort study, the Cancer Prevention Study II (CPS-II). Enrollees were usually friends, neighbors, or relatives of the volunteers; enrollment was by family groups with at least one person in the household 45 years of age or older. Subjects were asked to fill out a four-page confidential questionnaire and return it in a sealed envelope. The questionnaire included history of cancer and other diseases; use of medications and vitamins; menstrual and

reproductive history; occupational history; and information on diet, drinking, smoking, and other habits. The questionnaire also included three questions on occupation: (1) current occupation, (2) last occupation, if retired, and (3) job held for the longest period of time, if different from the other two. Occupations were coded to an ad hoc two-digit classification in 70 categories. Exposures at work or in daily life to any of the 12 groups of substances were also ascertained. These included diesel engine exhausts, asbestos, chemicals/acids/solvents, dyes, formaldehyde, coal or stone dusts, and gasoline exhausts. Volunteers checked whether their enrollees were alive or dead and recorded the date and place of all deaths every other year during the study. Death certificates were then obtained from state health departments and coded according to a system based on the ninth revision of the ICD by a trained nosologist.

The data were analyzed to determine the mortality for all causes and lung cancer in relation to diesel exhaust exposure, mortality for all causes and lung cancer and employment in selected occupations with high diesel exhaust exposure, and mortality from other causes in relation to diesel exhaust exposure. The incidence-density ratio was used as a measure of association, and test-based confidence limits were calculated by the Miettinen method. For stratified analysis, the Mantel-Haenszel method was used for testing linear trends. Data on 476,648 subjects comprising 939,817 person-years of risk were available for analysis. Three percent of the subjects (14,667) had not given any smoking history, and 20% (98,026) of them did not give information on diesel exhaust exposure and were therefore excluded from the main diesel exhaust analysis. Among individuals who had provided diesel exhaust exposure history, 62,800 were exposed and 307,143 were not exposed. Comparison of the population with known information on deisel exhaust exposure with the excluded population with no information on deisel exhaust exposure showed that the mean ages were 54.7 and 57.7 years, the nonsmokers were 72.4 and 73.2%, and the total mortality rates per 1,000 per year were 23.0 and 28.8%, respectively.

The all-cause mortality was elevated among railroad workers (relative risk [RR] = 1.43, 95% confidence interval [CI] = 1.2, 1.72), heavy equipment operators (RR = 1.7, 95% CI = 1.19, 2.44), miners (RR = 1.34, 95% CI = 1.06, 1.68), and truck drivers (RR = 1.19, 95% CI = 1.07, 1.31). For lung cancer mortality the risks were significantly elevated for miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators

(RR = 2.60, 95% CI = 1.12, 6.06). Risks were also elevated but not significantly for railroad workers (RR = 1.59, 95% CI = 0.94, 2.69) and truck drivers (RR = 1.24, 95% CI = 0.93, 1.66). These risks were calculated according to the Mantel-Haenszel method, controlling for age and smoking. Although the relative risk was nonsignificant for truck drivers, a small dose-response effect was observed when duration of diesel exhaust exposure for them was examined. For drivers who worked for 1 to 15 years, the relative risk was 0.87, while for drivers who worked for more than 16 years the relative risk was 1.33 (95% CI = 0.64, 2.75). Relative risks for lung cancer were not presented for other occupations. Mortality analysis for other causes and diesel exhaust exposure showed a significant excess of deaths ($p < 0.05$) in the following categories: cerebrovascular disease, arteriosclerosis, pneumonia, influenza, cirrhosis of the liver, and accidents.

The two main methodologic concerns in this study are the representativeness of the study population and the quality of information on exposure. The sample, though very large, was comprised of volunteers. Thus, the cohort was healthier and less frequently exposed to important risk factors such as smoking and alcohol. Self-administered questionnaires were used to obtain data on occupation and diesel exhaust exposure. None of this information was validated. Nearly 20% of the individuals had an unknown exposure status to diesel exhaust, and they experienced a higher mortality for all causes and lung cancer than both the diesel exhaust exposed and unexposed groups. This could have introduced a substantial bias in the estimate of the association. Although only 0.8% of the subjects were lost to follow-up, the use of death certificates alone as a source of medical information poses problems in accuracy and coding. But the authors report that cancer deaths are routinely checked by histological confirmation from physicians or cancer registries. Given the fact that all diesel exhaust exposure occupations, such as heavy equipment operators, truck drivers, and railroad workers showed elevated lung cancer risk, this study is suggestive of a causal association between the two.

### 8.2.7 Garshick et al. (1988): A Retrospective Cohort Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers

An earlier case-control study of lung cancer and diesel exhaust exposure in U.S. railroad workers by these investigators had demonstrated a relative odds of

1.41 (95% CI = 1.06, 1.88) for lung cancer with 20 years of work in jobs with diesel exhaust exposure. To confirm these results, a large retrospective cohort mortality study was conducted by the same investigators. Data sources for the study were the work records of the U.S. Railroad Retirement Board (RRB). The cohort was selected based on job titles in 1959, which was the year by which 95% of the locomotives in the United States were diesel powered. Diesel exhaust exposure was considered to be a dichotomous variable depending on yearly job codes between 1959 and death or retirement through 1980. Industrial hygiene evaluations and descriptions of job activities were used to classify jobs as exposed or unexposed to diesel emissions. A questionnaire survey of 534 workers at one of the railroads where workers were asked to indicate the amount of time spent in railroad locations, either near or away from sources of diesel exhaust, was used to validate this classification. Workers selected for this survey were actively employed at the time of the survey, 40 to 64 years of age, who started work between 1939 and 1949, in the job codes sampled in 1959, and were eligible for railroad benefits. To qualify for benefits, a worker must have 10 years or more of service with the railroad and should not have worked for more than 2 years in a nonrailroad job after leaving railroad work. Workers with recognized asbestos exposure, such as repair of asbestos-insulated steam locomotive boilers, passenger cars, and steam pipes, or railroad building construction and repairs were excluded from the job categories selected for study. However, a few jobs with some potential for asbestos exposure were included in the cohort, and the analysis was done both ways with and without them.

The death certificates for all subjects identified in 1959 and reported by the RRB to have died through 1980 were searched. Twenty-five percent of them were obtained from the RRB and the remainder from the appropriate state departments of health. Coding of cause of death was done without knowledge of exposure history, according to the eighth revision of the ICD. If the underlying cause of death was not lung cancer, but was mentioned on the death certificate, it was assigned as a secondary cause of death, so that the ascertainment of all cases was complete. Workers not reported by the RRB to have died by December 31, 1980, were considered to be alive. Deceased workers for whom death certificates had not been obtained, or if obtained did not indicate cause of death, were assumed to have died of unknown causes.

Proportional hazard models were fitted that provided estimates of relative risk for death caused by lung cancer using the partial likelihood method described by Cox, and 95% confidence intervals were constructed using the asymptotic normality of the estimated regression coefficients of the proportional hazards model. Exposure was analyzed by diesel exhaust-exposed jobs in 1959 and by cumulative number of years of diesel exhaust exposure through 1980. Directly standardized rate ratios for deaths from lung cancer were calculated for diesel exhaust exposed compared with unexposed for each 5-year age group in 1959. The standardized rates were based on the overall 5-year person-year time distribution of individuals in each age group starting in 1959. The only exception to this was between 1979 and 1980, when a 2-year person-year distribution was used. The Mantel-Haenszel analogue for person-year data was used to calculate 95% confidence intervals for the standardized rate ratios.

The cohort consisted of 55,407 workers, of which 19,396 had died by the end of 1980. Death certificates were not available for 11.7% of all deaths. Of the 17,120 deaths for whom death certificates were obtained, 48.4% were attributable to diseases of the circulatory system, whereas 21% were attributable to all neoplasms. Of all neoplasms, 8.7% (1,694 deaths) were due to lung cancer. A higher proportion of workers in the younger age groups, mainly brakemen and conductors, were exposed to diesel exhaust, while a higher age of workers in the older age groups were potentially exposed to asbestos. In a proportional hazards model, analyses by age in 1959 found a relative risk of 1.45 (95% CI = 1.11, 1.89) among the age group 40 to 44 years and a relative risk of 1.33 (95% CI = 1.03, 1.73) for the age group 45 to 49 years. Risk estimates in the older age groups 50 to 54, 55 to 59, and 60 to 64 years were 1.2, 1.18, and 0.99, respectively, and were not statistically significant. The two youngest age groups in 1959 had workers with the highest prevalence and longest duration of diesel exhaust exposure and lowest exposure to asbestos. When potential asbestos exposure was considered as a confounding variable in a proportional hazards model, the estimates of relative risk for asbestos exposure were all near null value and not significant. Analysis of workers exposed to diesel exhaust in 1959 (n = 42,535), excluding the workers with potential past exposure to asbestos, yielded relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) in the 1959 age groups 40 to 44 years and 45 to 49 years. Directly standardized rate ratios were also calculated for each

1959 age group based on diesel exhaust exposure in 1959. The results obtained confirmed those obtained by using the proportional hazards model.

Relative risk estimates were then obtained using duration of diesel exhaust exposure as a surrogate for dose. In a model that used years of exposure up to and including exposure in the year of death, no exposure duration-response relationship was obtained. When analysis was done by disregarding exposure in the year of death and 4 years prior to death, the risk of dying from lung cancer increased with the number of years worked in a diesel exhaust-exposed job. In this analysis, exposure to diesel exhaust was analyzed by exposure duration groups and in a model entering age in 1959 as a continuous variable. The workers with greater than 15 years of exposure had a relative risk of lung cancer of 1.72 (95% CI = 1.27, 2.33). The risks for 1 to 4 years of cumulative exposure was 1.20 (95% CI = 1.01, 1.44), for 5 to 9 years of cumulative exposure it was 1.24 (95% CI = 1.06, 1.44), and for 10 to 14 years of cumulative exposure it was 1.32 (95% CI = 1.13, 1.56). Directly standardized rate ratios were also calculated for each 1959 age group based on diesel exposure in 1959. The results obtained confirmed those obtained by using the proportional hazards model.

The results of this study, demonstrating a positive association between diesel exhaust exposure and increased lung cancer, are consistent with the results of the case-control study conducted by the same investigators in railroad workers dying of lung cancer from March 1981 through February 1982. This cohort study has addressed many of the weaknesses of the other epidemiologic studies. The large sample size (60,000) allowed sufficient power to detect small risks and also permitted the exclusion of workers with potential past exposure to asbestos. The stability of job career paths in the cohort ensured that of the workers 40 to 44 years of age in 1959 classified as diesel exhaust- exposed, 94% of the cases were still in diesel exhaust-exposed jobs 20 years later.

The main limitation of the study is the lack of quantitative data on exposure to diesel exhaust. This is one of the few studies in which industrial hygiene measurements of diesel exhaust were done. These measurements were correlated with job titles to divide the cohort in dichotomous exposure groups of exposed and nonexposed. This may have lead to an underestimation of the risk of lung cancer since exposed groups included individuals with low to high exposure. The number of years exposed to diesel exhaust was used as a surrogate for dose. The dose, based on duration of employment, may have been inaccurate because

individuals were working on steam or diesel locomotives during the transition period. If the categories of exposure to diesel exhaust would have been set up as no, low, moderate, and high exposure, the results would have been more meaningful and so would have been the dose-response relationship. Another limitation of this study was the inability to examine the effect of years of exposure and latency. No adjustment for smoking was done in this study. However, an earlier case-control study done in the same cohort (Garshick et al., 1987) showed no significant difference in the risk estimate after adjusting for smoking. Despite these limitations, the results of this study demonstrate that occupational exposure to diesel exhaust is associated with a modest risk (1.5) of lung cancer.

Table 8-1 summarizes the cohort studies.

## 8.3 CASE-CONTROL STUDIES OF LUNG CANCER

### 8.3.1 Williams et al. (1977): Associations of Cancer Site and Type with Occupation and Industry from the Third National Cancer Survey Interview

This paper reports findings of the analysis of the Third National Cancer Survey (TNCS). The lifetime histories, occupations, and industries were studied for associations with specific cancer sites and types after controlling for age, sex, race, education, use of cigarettes or alcohol, and geographic location. Of 13,179 cancer patients, a 10% random sample of all incident invasive cancers in eight areas, a total of 7,518 were successfully interviewed in the 3 years surveyed by the TNCS. These comprised 57% of those eligible to participate. The interview included items on use of tobacco and alcohol (by type, amount, and duration), family income, patient education, and employment history. Actual descriptions of the occupation and industry were recorded by interviewers and were coded separately for main lifetime employment, recent employment, and other jobs held according to the 1970 Census Coding Scheme. Occupations or industries were combined to form larger groups. Coding of occupational and industrial labels in meaningful job categories was done by one of the authors. Of the 3,539 interviewed males and 3,937 interviewed females, 95 and 84%, respectively, listed some main employment. The basic analysis consisted of an intercancer comparison and involved comparing the proportions of specific main lifetime

## TABLE 8-1. EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: COHORT MORTALITY STUDIES

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Waller (1981) | Approximately 20,000 male London transportation workers<br><br>Aged 45 to 64 years<br><br>25 years follow-up (1950-1974) | Five job categories used to define exposure<br><br>Environmental benzo[*a*]pyrene concentrations measured in 1957 and 1979 | SMR = 79 for lung cancer for the total cohort<br><br>SMRs for all five job categories were less than 100 for lung cancer. | Exposure measurement of benzo[*a*]pyrene showed very little difference between inside and outside the garage.<br><br>Incomplete information on cohort members<br><br>No adjustment for confounding such as other exposures, cigarette smoking, etc.<br><br>No latency analysis |
| Howe et al. (1983) | 43,826 Male pensioners of the Canadian National Railway Company<br><br>Mortality between 1965 and 1977 among these pensioners was compared with mortality of general Canadian population. | Exposure groups classified by a group of experts based on occupation at the time of retirement<br><br>Three exposures groups:<br>Nonexposed<br>Possibly exposed<br>Probably exposed | RR = 1.2 ($p = 0.013$)<br>RR = 1.3 ($p = 0.001$) for lung cancer for possible and probable exposure, respectively<br><br>A highly significant dose-response relationship demonstrated by trend test ($p < 0.001$) | Incomplete exposure assessment due to lack of lifetime occupational history<br><br>Mixed exposures to coal dust and diesel exhaust<br><br>No validation of method was used to categorize exposure.<br><br>No data on smoking<br><br>No latency analysis |
| Rushton et al. (1983) | 8,490 Male London transport maintenance workers<br><br>Mortality of workers employed for 1 continuous year between January 1, 1967, and December 31, 1975, was compared with mortality of general population of England and Wales. | 100 Different job titles were grouped in 20 broad categories.<br><br>The categories were not ranked for diesel exhaust exposure. | SMR = 133 ($p < 0.03$) for lung cancer in the general hand job group<br><br>Several other job categories showed SS increased SMRs for several other sites based on less than five cases. | Ill-defined diesel exhaust exposure without any ranking<br><br>Average 6-year follow-up, (i.e. not enough time for lung cancer latency)<br><br>No adjustment for confounders such as smoking |

**TABLE 8-1 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: COHORT MORTALITY STUDIES**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Wong et al. (1985) | 34,156 Male heavy construction equipment operators<br><br>Members of the local union for at least 1 year between January 1, 1964, and December 1, 1978 | 20 Functional job titles grouped into three job categories for potential exposure<br><br>Exposure groups (high, low, and unknown) based on job description and proximity to source of diesel exhaust emissions | SMR = 166 ($p < 0.05$) for liver cancer for total cohort<br><br>SMR = 343 (observed = 5, $p < 0.05$) for lung cancer for high exposure bulldozer operators with 15-19 years of membership, 20+ years of follow-up<br><br>SMR = 119 (observed = 141, $p < 0.01$) for workers with no work histories | No validation of exposure categories, which were based on surrogate information<br><br>Incomplete employment records<br><br>Employment history other than from the union not available<br><br>No data on confounder such as other exposures, smoking, etc. |
| Edling et al. (1987) | 694 Male bus garage employees<br><br>Follow-up from 1951 through 1983<br><br>Mortality of these men was compared with mortality of general population of Sweden. | Three exposure groups based on job titles:<br>High exposure, bus garage workers<br>Intermediate exposure, bus drivers<br>Low exposure, clerks | No SS differences were observed between observed and expected for any cancers by different exposure groups. | Small sample size<br><br>No validation of exposure<br><br>No data on confounders such as other exposures, smoking, etc. |



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**TABLE 8-1 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: COHORT MORTALITY STUDIES**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Boffetta and Stellman (1988) | 46,981 Male volunteers enrolled in the American Cancer Society's Prospective Mortality Study of Cancer in 1982<br><br>Aged 40 to 79 years at enrollment<br><br>First 2-year follow-up | Self-reported occupations were coded into 70 job categories.<br><br>Employment in high diesel exhaust exposure jobs were compared with nonexposed jobs. | Total mortality (SS) elevated for railroad workers, heavy equipment operators, miners, and truck drivers<br><br>Lung cancer mortality (SS) elevated for miners and heavy equipment operators<br><br>Lung cancer mortality (SNS) elevated among railroad workers and truck drivers<br><br>Truck drivers also showed a small dose response | Exposure information based on self-reported occupation for which no validation was done<br><br>Volunteer population, probably healthy population |
| Garshick et al. (1988) | 55,407 White male railroad workers<br><br>Aged 40 to 64 years in 1959<br><br>Started work 10-20 years earlier than 1959 | Industrial hygiene data correlated with job titles to dichotomize the jobs as "exposed" or "not exposed" | RR = 1.45 (40-44 year age group)<br>RR = 1.33 (45-49 year age group)<br>Both SS<br><br>After exclusion of workers exposed to asbestos<br>RR = 1.57 (40-44 year age group)<br>RR = 1.34 (45-49 year age group)<br>Both SS<br><br>Dose response indicated by increasing lung cancer risk with increasing cumulative exposure | Years of exposure used as surrogate for dose<br><br>Not possible to separate the effect of time since first exposure and duration of exposure |

Abbreviations:
RR = Relative risk.
SMR = Standardized mortality ratio.
SNS = Statistically nonsignificant.
SS = Statistically significant.



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industries and occupations among patients with cancer at one site with those of patients having cancer at other sites combined as a control group, and this was done using a series of Mantel-Haenszel stratified contingency table analyses to yield odds ratios and chi-square values. Odds ratios (ORs) were computed controlling for age, race, education, tobacco, alcohol, and geographic location, and these were done separately for males and females.

A total of 432 and 128 lung cancers were present in males and females, respectively. For males an excess risk of lung cancer was observed for the following main industrial groups: mines (OR = 1.21), construction (OR = 1.24), transportation (OR = 1.17), utility and sanitary services (OR = 2.79, $p < 0.05$), and professional (OR = 1.41). An excess of bladder cancer was reported for the mining industry (OR = 1.61). For females, an excess of lung cancer was detected for the transportation industry (OR = 1.96); finance and retail industry (OR = 1.73); and the business, car repair, and miscellaneous service industry (OR = 2.29). None of these excesses were statistically significant. All these odds ratios were adjusted for age, race, education, tobacco, alcohol, and geographic location. The transportation industry for males and females also showed a nonsignificant excess risk for cancers of the liver and gall bladder ducts. When the analysis was done for specific lifetime industries, the odds ratios for lung cancer in males was 1.40 for railroad workers and 1.34 for truck drivers. Both these excesses were statistically nonsignificant.

The strengths of the TNCS interview data set are its large size, histological confirmation of nearly 95% of diagnoses, availability of information on occupation, and details of confounding variables obtained by personal interview and ability to control for them. Among its weaknesses are a 47% nonresponse rate and the fact that the population surveyed came from predominantly urban areas and did not represent many industries. Also, most of the associations observed did not achieve statistical significance because they were based on small numbers of patients who had both specific cancers and specific types of employment. The control group was the combined "other cancers" which may have diluted the association since diesel exhaust is also suspected of being associated with bladder cancer, and this category was included in the control group when the comparison was made with lung cancer. The study presented several tables, but the total population in each table was different and never added up to the initial number interviewed. The authors failed to explain these omissions. Further, when multiple comparisons are made, some significant

associations arise by chance. This analysis does suggest an association with lung cancer for three industries with potential diesel exhaust exposure. These were trucking, railroading, and mining.

### 8.3.2 Hall and Wynder (1984): A Case-Control Study of Diesel Exhaust Exposure and Lung Cancer

Hall and Wynder conducted a case-control study of 502 male lung cancer cases and 502 controls without tobacco-related diseases that examined an association between occupational diesel exhaust exposure and lung cancer. Histologically confirmed primary lung cancer patients who were 20 to 80 years old were ascertained from 18 participating hospitals in six U.S. cities, 12 mo prior to the interview. Eligible controls, comprised patients at the same hospitals without tobacco-related diseases, were matched to cases by age ($\pm$5 years), race, hospital, and hospital room status. The number of male lung cancer cases interviewed totaled 502, which was 64% of those who met the study criteria for eligibility. Of the remaining 36%, 8% refused, 21% were too ill or had died, and 7% were unreliable. Seventy-five percent of eligible controls completed interviews. Of these interviewed controls, 49.9% were from the all cancers category, whereas 50.1% were from the all noncancers category. All interviews were obtained in hospitals to gather detailed information on smoking history, coffee consumption, artificial sweetener use, residential history, and abbreviated medical history as well as standard demographic variables. Occupational information was elicited by a question on the usual lifetime occupation and was coded by the abbreviated list of the U.S. Bureau of Census Codes. The odds ratios were calculated to evaluate the association between diesel exhaust exposure and risk of lung cancer incidence. Summary odds ratios were computed by the Mantel-Haenszel method after adjusting for potential confounding by age, smoking, and socioeconomic class. Two sided, 95% confidence intervals were computed by Woolf's method. Occupational exposure to diesel exhaust was defined by two criteria. First, occupational titles were coded "probably high exposure" as defined by the industrial hygiene standards established for the various jobs. The job titles included under this category were warehousemen, bus and truck drivers, railroad workers, and heavy equipment operators and repairmen. The second method used the National Institute for Occupational Safety and Health (NIOSH) criteria to analyze

occupations by diesel exposure. In this method, the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10 to 19% of their employees exposed were defined as "moderate exposure," and those with less than 10% of their empolyees exposed were defined as "low exposure."

Cases and controls were compared with respect to exposure. The relative risk was 2.0 (95% CI = 1.2, 3.2) for those workers who were exposed to diesel exhaust versus those who were not. The risk, however, decreased to a nonsignificant 1.4 when the data were adjusted for smoking. Analysis by NIOSH criteria found a nonsignificant relative risk of 1.7 in the high exposure group. There were no significantly increased cancer risks by occupation either by the first method or by the NIOSH method. In order to assess any possible synergism between diesel exhaust exposure and smoking, the lung cancer risks were calculated for different smoking categories. The relative risks were 1.46 among nonsmokers and exsmokers, 0.82 among current smokers of $\leq$20 cigarettes/day, and 1.3 among current smokers of 20+ cigarettes/day indicating a lack of synergistic effects.

The major strength of this study is the availability of a detailed smoking history for all the study subjects. However, this is offset by the lack of diesel exhaust exposure measurements, use of a poor surrogate for exposure, and lack of consideration of latency period. Information was collected on only one major lifetime occupation, and it is likely that those workers who had more than one major job may not have reported the occupation with the heaviest diesel exhaust exposures. Further, occupational histories were obtained from self reports and were not validated with work records. This could have resulted in recall bias and misclassification of exposure status.

### 8.3.3 Damber and Larsson (1987): Occupation and Male Lung Cancer, a Case-Control Study in Northern Sweden

A case-control study of lung cancer was conducted in northern Sweden to determine the occupational risk factors that could explain the large geographic variations of lung cancer incidence in that country. The study region comprised the three northern-most counties of

Sweden with a total male population of about 390,000. The rural municipalities with 15 to 20% of the total population have forestry and agriculture as dominating industries and the urban areas have a variety of industrial activities (mines, smelters, steel factories, paper mills, and mechanical workshops). All male cases of lung cancer reported to the Swedish Cancer Registry during the 6-year period between 1972 and 1977, who had died before the start of the study, were selected. Of 604 eligible cases, 5 did not have microscopic confirmation and in another 5 the diagnosis was doubtful, but these cases were included nevertheless. Cases were classified as small carcinomas, squamous cell carcinomas, adenocarcinomas, and other types. For each case a dead control was drawn from the National Death Registry matched by sex, year of death, age, and municipality. Deaths in controls classified as lung cancer and suicides were excluded. A living control matched to the case by sex, year of birth, and municipality was also drawn from the National Population Registry. Postal questionnaires were sent to close relatives of cases and dead controls, and to living controls themselves to collect data on occupation, employment, and smoking habits. Replies were received from 589 cases (98%) 582 surrogates of dead controls (96%), and 453 living controls (97%).

Occupational data were collected on occupations or employment held for at least 1 year and included type of industry, company name, task, and duration of employment. Supplementary telephone interviews were performed if occupational data were lacking for any period between age 20 and time of diagnosis. Data analysis involved calculation of the odds ratios by the exact method based on the hypergeometric distribution and the use of a linear logistic regression model to adjust for the potential confounding effects of smoking. Separate analyses were performed with dead and living controls, and on the whole there was good agreement between the two control groups. A person who had been active for at least 1 year in a specific occupation was in the analysis assigned to that occupation.

Using dead controls, the odds ratios adjusted for smoking were 1.0 (95% CI = 0.7, 1.5) and 2.7 (95% CI = 1.0, 8.1) for professional drivers ($\geq$1 year of employment) and underground miners ($\geq$1 year of employment), respectively. For 20 or more years of employment in those occupations, the odds ratios adjusted for smoking were 1.2 (95% CI = 0.6, 2.2) and 9.8 (95% CI = 1.5, 414). These were the only two occupations listed with potential diesel exhaust exposure. An excess significant risk was

detected for copper smelter workers, plumbers, and electricians, as well as concrete and asphalt workers. Occupational asbestos exposure was also associated with an elevated odds ratio of 2.6 (95% CI = 1.6, 3.6) for ≥1 year of employment and 3.6 (95% CI = 1.9, 7.2) for ≥20 years of employment. All the odds ratios were calculated by adjusting for age, smoking, and municipality. After comparison with the live controls, the odds ratios were found to be lower than those observed with dead controls. None of the odds ratios were statistically significant in this comparison.

This study did not detect any excess risk of lung cancer for professional drivers who, among all the occupations listed, had the most potential for exposure to motor vehicle exhaust. However, it is not known whether these drivers were exposed exclusively to gasoline exhaust, diesel exhaust, or varying degrees of both. An excess risk was detected for underground miners, but it is not known if this was due to diesel emissions from engines or from radon daughters in poorly ventilated mines. Although a high response rate (98%) was obtained by the postal questionnaires, the use of surrogate respondents is known to lead to misclassification errors that can bias the odds ratio to one.

### 8.3.4 Lerchen et al. (1987): Lung Cancer and Occupation in New Mexico

This is a population-based case-control study conducted in New Mexico that examines the association between occupation and occurrence of lung cancer in Hispanic and non-Hispanic whites. Cases involved residents of New Mexico, 25 through 84 years of age and diagnosed between January 1, 1980, and December 31, 1982, with primary lung cancer, excluding bronchioalveolar carcinoma. Cases were ascertained through the New Mexico Tumor Registry which is a member of the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute. Controls were chosen by randomly selecting residential telephone numbers, and for those over 65 years of age, from the Health Care Financing Administration's roster of Medicare participants. They were frequency-matched to cases for sex, ethnicity, and 10-year age category with a ratio of 1.5 controls per case. The 506 cases (333 males and 173 females) and 771 controls (499 males and 272 females) were interviewed, with a nonresponse rate of 11% for cases. Next of kin provided interviews for 50 and 43% of male and female cases, respectively. Among controls, only 2% of the interviews were provided by next of kin for each sex. Data were

collected by personal interviews conducted by bilingual interviewers in the participants' homes. A lifetime occupational history and a self-reported history of exposure to specific agents were obtained for each job held for at least 6 mo since age 12. Questions were asked about the title of the position, duties performed, location and nature of industry, and time at each job title. A detailed smoking history was also obtained. The variables on occupational exposures were coded according to the Standard Industrial Classification scheme by a single person and reviewed by another. To test the hypothesis about the high-risk jobs for lung cancer, an a priori listing of suspected occupations and industries was created by a two-step process involving a literature review for implicated industries and occupations by the principal investigator. The appropriate Standard Industrial Classification and Standard Occupational Codes associated with job titles were also determined by the principal investigator. For four agents, asbestos, wood dust, diesel exhaust, and formaldehyde, the industries and occupations determined to have exposure were identified, and linking of specific industries and occupations was based on literature review and consultation with local industrial hygienists.

The relative odds were calculated for suspect occupations and industries classifying individuals as ever employed for at least 1 year in an industry or occupation and defining the reference group as those subjects never employed in that particular industry or occupation. Multiple logistic regression models were used to control simultaneously for age, ethnicity, and smoking status. For occupations with potential diesel exhaust exposure, the analysis showed no excess risks for diesel engine mechanics and auto mechanics. Similarly, when analyzed by exposure to specific agents, the odds ratio adjusted for age, smoking, and ethnicity was not elevated for diesel exhaust fumes (OR = 0.6, 95% CI = 0.2, 1.6). Elevated odds ratios were found for uranium miners (OR = 2.8, 95% CI = 1.0, 7.7), underground miners (OR = 2.4, 95% CI = 1.2, 4.4), construction painters (OR = 2.4, 95% CI = 0.6, 9.6), and welders (OR = 4.3, 95% CI = 1.6, 11.0). No excess risks were detected for the following industries: shipbuilding, petroleum refining, construction, printing, blast furnace, and steel mills; or for the following occupations: construction workers, painters, plumbers, paving equipment operators, roofers, engineers and firemen, woodworkers, and shipyard workers. Females were excluded from detailed analysis because none of the Hispanic female controls had been employed in high-risk jobs; among the

non-Hispanic white controls, employment in a high-risk job was recorded for at least five controls for only two industries, construction and painting, for which the odds ratios were not significantly elevated. Therefore, the analyses were presented for males only.

Among the many strengths of this study are its population-based design, high participation rate, detailed smoking history, and the separate analysis done for the two ethnic groups, southwestern Hispanic and non-Hispanic white males. The major limitations pertain to the occupational exposure date. Job titles obtained from occupational histories were used as proxy for exposure status, but these were not validated. Further, for nearly half the cases, next of kin provided occupational histories. The authors acknowledge the above sources of bias but state without substantiation that these biases would not strongly affect their results. They also did not use a job exposure matrix to link occupations to exposures and did not provide details on the method they used to classify individuals as diesel exhaust-exposed based on reported occupations. The observed absence of an association for exposure to asbestos, a well-established lung carcinogen, may be explained by the misclassification errors in exposure status or by sample size constraints (not enough power). Likewise, the association for diesel exhaust reported by only 7 cases and 17 controls also may have gone undetected because of low power. In conclusion, there is insufficient evidence from this study to confirm or refute an association between lung cancer and diesel exhaust exposure.

### 8.3.5 Garshick et al. (1987): A Case-Control Study of Lung Cancer and Diesel Exhaust Exposure in Railroad Workers

An earlier pilot study of the mortality of railroad workers by the same investigators (Schenker et al., 1984) found a moderately high risk of lung cancer among the workers who were exposed to diesel exhaust as compared to those who were not. This study was designed to evaluate the feasibility of conducting a large retrospective cohort study. On the basis of these findings the investigators conducted a case-control study of lung cancer in the same population. The population base for this case-control study of lung cancer and diesel exhaust was approximately 650,000 active and retired male U.S. railroad workers with 10 years or more of railroad service who were born in 1900 or later. The U.S. Railroad Retirement Board (RRB), which operates the retirement system, is separate from the Social Security System, and to qualify for the retirement or survivor benefits the workers had to acquire

10 years or more of service. Information on deaths that occurred between March 1, 1981, and February 28, 1982, was obtained from the RRB. For 75% of the deceased population, death certificates were obtained from the RRB, and, for the remaining 25%, they were obtained from the appropriate state departments of health. Cause of death was coded according to the eighth revision of the ICD. The cases were selected from deaths with primary lung cancer, which was the underlying cause of death in most cases. Each case was matched to two deceased controls whose dates of birth were within 2.5 years of the date of birth of the case and whose dates of death were within 31 days of the date of death noted in the case. Controls then were selected randomly from workers who did not have cancer noted anywhere on their death certificates and who did not die of suicide or of accidental or unknown causes.

Each subject's work history was determined from a yearly job report filed by his employer with the RRB from 1959 until death or retirement. The year 1959 was chosen as the effective start of diesel exhaust exposure for this study, since by this time 95% of the locomotives in the United States were diesel powered. Investigators acknowledge that because the transition to diesel-powered engines took place in the early 1950s, some workers had additional exposure prior to 1959; however, if a worker had died or retired prior to 1959, he was considered unexposed. Exposure to diesel exhaust was considered to be dichotomous for this study, which was assigned based on an industrial hygiene evaluation of jobs and work areas. Selected jobs with and without regular diesel exhaust exposure were identified by a review of job title and duties. Personal exposure was assessed in 39 job categories representative of workers with and without diesel exhaust exposure. Those jobs for which no personal sampling was done were considered exposed or unexposed on the basis of similarities in job activities and work locations and by degree of contact with diesel equipment. Asbestos exposure was categorized on the basis of jobs held in 1959 or on the last job held if the subject retired before 1959. Asbestos exposure in railroads occurred primarily during the steam engine era and was related mostly to the repair of locomotive steam boilers that were insulated with asbestos. Smoking history information was obtained from the next of kin.

Death certificates were obtained for approximately 87% of the 15,059 deaths reported by the RRB from which 1,374 cases of lung cancer were identified. Fifty-five cases of lung

cancer were excluded from the study for either incomplete data (20) or refusal by two states to use information on death certificates to contact the next of kin. Successful matching to at least one control with work histories was achieved for 335 (96%) cases ≤64 years of age at death and 921 (95%) cases ≥65 years of age at death. In both age groups, 90% of the cases were matched with two controls. There were 2,385 controls in the study, 98% were matched within ±31 days of the date of death, whereas the remaining 2% were matched within 100 days. Deaths from diseases of the circulatory system predominated among controls. Among the younger workers, approximately 60% had exposure to diesel exhaust, whereas, among older workers, only 47% were exposed to diesel exhaust.

Analysis by a regression model, in which years of diesel exhaust exposure was the sum total of the number of years in diesel-exposed jobs, used as a continuous exposure variable, yielded an odds ratio of lung cancer to be 1.39 (95% CI = 1.05, 1.83) for over 20 years of diesel exhaust exposure in the ≤64 years of age group. After adjustment for asbestos exposure and lifetime smoking (pack-years), the odds ratio was 1.41 (95% CI = 1.06, 1.88). Both crude odds ratio and asbestos exposure as well as lifetime smoking adjusted odds ratio for the ≥65 years of age group were not significant. Increasing years of diesel exhaust exposure categorized as ≥20 diesel years and 5 to 19 diesel years with 0 to 4 years as the referent group showed significantly increased risk in the ≤64 years of age group after adjusting for asbestos exposure and pack-year category of smoking. For individuals who had ≥20 years of diesel exhaust exposure, the odds ratio was 1.64 (95% CI = 1.18, 2.29), whereas individuals who had 5 to 19 years of diesel exhaust exposure, the odds ratio was 1.02 (95% CI = 0.72, 1.45). In the ≥65 years of age group, only 3% of the workers were exposed to diesel exhaust for more than 20 years. Relative odds for 5 to 19 years and ≥20 years of diesel exposure were less than 1 ($p > 0.01$), after adjusting for smoking and asbestos exposure.

Alternate models to explain post-asbestos exposure were tested. These were variables for regular and intermittent exposure groups and an estimate of years of exposure based on estimated years worked prior to 1959. No difference in results were seen. The interaction between diesel exhaust exposure and the three pack-year categories ([1] <50, [2] >50, and [3] missing pack-years) were explored. The cross-product terms were not significant. A model was also tested that excluded recent diesel exhaust exposure occurring within the

5 years before death and gave an odds ratio of 1.43 (95% CI = 1.06, 1.94) adjusted for cigarette smoking and asbestos exposure, for workers with 15 years of cumulative exposure. For workers with 5 to 14 years of cumulative exposure, the relative odds were not significant.

The many strengths of the study are consideration of confounding factors such as asbestos exposure and smoking; classification of diesel exhaust exposures by job titles and industrial hygiene sampling; exploration of interactions between smoking, asbestos exposure, and diesel exhaust exposure; and good ascertainment (87%) of death certificates from the 15,059 deaths reported by the RRB.

The investigators also recognized and reported the following limitations: overestimation of cigarette consumption by surrogate respondents which may have exaggerated the contribution of smoking to lung cancer risk and use of the Interstate Commerce Commission (ICC) job classification as a surrogate for exposure which may have lead to misclassification of diesel exhaust exposure jobs with low intensity and intermittent exposure, such as railroad police and bus drivers, as unexposed. These two limitations would result in the underestimation of the lung cancer risk. This source of error could have been avoided if diesel exhaust exposures were categorized by a specific dose range associated with a job title that could have been classified as heavy, medium, low, and zero exposure instead of a dichotomous variable. The use of death certificates to identify cases and controls may have resulted in misclassification. Controls may have had undiagnosed primary lung cancer, and lung cancer cases might have been secondary lesions misdiagnosed as primary lung cancer. However, the investigators quote a third National Cancer Survey report in which the death certificates for lung cancer were coded appropriately in 95% of the cases. Lastly, as in all previous studies, there is a lack of data on the contribution of unknown occupational or environmental exposures and passive smoking. In conclusion, this study, compared with previous studies (on diesel exposure and lung cancer risk), provides the most valid evidence that occupational diesel exhaust emission exposure increases the risk of lung cancer.

### 8.3.6 Benhamou et al. (1988): Occupational Risk Factors of Lung Cancer in a French Case-Control Study

This is a case-control study of 1,625 histologically confirmed cases of lung cancer and 3,091 matched controls, conducted in France between 1976 and 1980. This study was part of an international study to investigate the role of smoking and lung cancer. Each case was matched with one or two controls whose diseases were not related to tobacco use, sex, age at diagnosis ($\pm 5$ years), hospital of admission, or interviewer. Information was obtained from both cases and controls on place of residence since birth, educational level, smoking, and drinking habits. A complete lifetime occupational history was obtained by asking participants to give their occupations from the most recent to the first. Women were excluded because most of them had listed no occupation. Men who smoked cigars and pipes were excluded because there were very few in this category. Thus, the study was restricted to nonsmokers and cigarette smokers. Cigarette smoking exposure was defined by age at the first cigarette (nonsmokers, $\leq 20$ years, or $> 20$ years), daily consumption of cigarettes (nonsmokers, $< 20$ cigarettes a day, and $\geq 20$ cigarettes a day), and duration of cigarette smoking (nonsmokers, $< 35$ years, and $\geq 35$ years). The data on occupations were coded by a panel of experts according to their own chemical or physical exposure determinations. Occupations were recorded blindly using the International Standard Classification of Occupations. Data on 1,260 cases and 2,084 controls were available for analysis. The remaining 365 cases and 1,007 controls were excluded because they did not satisfy the required smoking status criteria.

A matched logistic regression analysis was performed to estimate the effect of each occupational exposure after adjusting for cigarette status. Matched relative risk (RR) ratios were calculated for each occupation with the baseline category, which consisted of patients who had never been engaged in that particular occupation. The matched relative risk ratios adjusted for cigarette smoking for the major groups of occupations showed that the risks were significantly higher for production and related workers, transport equipment operators, and laborers (RR = 1.24, 95% CI = 1.04, 1.47). On further analysis of this group, for occupations with potential diesel emission exposure, significant excess risks were found for motor vehicle drivers (RR = 1.42, 95% CI = 1.07, 1.89) and transport equipment operators (RR = 1.35, 95% CI = 1.05, 1.75). No interaction with smoking status was found in any

of the occupations. The only other significant excess was observed for mines and quarrymen (RR = 2.14, 95% CI = 1.07, 4.31). None of the significant associations showed a dose-response relationship with duration of exposure.

This study was designed primarily to investigate the relationship between smoking (not occupations and environmental exposures) and lung cancer. Although an attempt was made to obtain complete occupational histories, the authors did not clarify if in the logistic regression analysis, they used the subjects first occupation, predominant occupation, last occupation, or ever worked in that occupation as the risk factor of interest. The most important limitation of this study is that the occupations were not coded into exposures for different chemical and physical agents, thus precluding the calculation of relative risks for diesel exposure. Using occupations as surrogate measures of diesel exposure, an excess significant risk was obtained for motor vehicle drivers and transport equipment operators, but not for motor mechanics. However, it is not known if subjects in these occupations worked with diesel engines or nondiesel engines.

### 8.3.7 Hayes et al. (1989): Lung Cancer in Motor Exhaust-Related Occupations

This study reports the findings from an analysis of pooled data from three lung cancer case-control studies that examine in detail the association between employment in motor exhaust-related (MER) occupations and lung cancer risk adjusted for confounding by smoking and other risk factors. The three studies were carried out by the National Cancer Institute in Florida (1976 to 1979), New Jersey (1980 to 1981), and Louisiana (1979 to 1983). These three studies were selected because the combined group would provide a sufficient sample to detect a risk of lung cancer in excess of 50% among workers in MER occupations. The analyses were restricted to males who had given occupational history. The Florida study was hospital-based with cases ascertained through death certificates. Controls were randomly selected from hospital records and death certificates, excluding psychiatric diseases matched by age and county. The New Jersey study was population-based with cases ascertained through hospital records, cancer registry, and death certificates. Controls were selected from among the pool of New Jersey licensed drivers and death certificates. The Louisiana study was hospital-based (it is not specified how the cases were ascertained), and controls were

randomly selected from hospital patients, excluding those with lung diseases and tobacco-related cancers.

A total of 2,291 cases of male lung cancers and 2,570 controls were eligible, and the data on occupations were collected by next-of-kin interviews for all jobs held for 6 mo or more, including the industry, occupation, and number of years employed. The proportion of next-of-kin interviews varied by site between 50% in Louisiana to 85% in Florida. The coding schemes were reviewed to identify MER occupations, which included truck drivers and heavy equipment operators (cranes, bulldozers, and graders); bus drivers, taxi drivers, chauffeurs, and other motor vehicle drivers; and automobile and truck mechanics. Truck drivers were classified as routemen and delivery men and other truck drivers. All jobs were also classified with respect to potential exposure to known and suspected lung carcinogens. Odds ratios were calculated by the maximum likelihood method adjusting for age by birth year, usual amount smoked, and study area. Logistic regression models were used to examine the interrelationship of multiple variables.

A statistically significant excess risk was detected for employment of 10 years or more for all MER occupations (except truck drivers) adjusted for birth cohort, usual daily cigarette use, and study area. The odds ratio for lung cancer using data gathered by direct interviews was 1.4 (95% CI = 1.1, 2.0), allowing for multiple MER employment and 2.0 (95% CI = 1.3, 3.0) excluding individuals with multiple MER employment. Odds ratios for all MER employment, except truck drivers who were employed for less than 10 years, were 1.3 (95% CI = 1.0, 1.7) and 1.3 (95% CI = 0.9, 1.8) for including and excluding multiple MER employment, respectively. Odds ratios were then derived for specific MER occupations, and, to avoid the confounding effects of multiple MER job classifications, analyses were also done excluding subjects with multiple MER job exposures. Truck drivers employed for more than 10 years had an odds ratio of 1.5 (95% CI = 1.1, 1.9). A similar figure was obtained excluding subjects with multiple MER employment. An excess risk was not detected for truck drivers employed less than 10 years. The only other job category that showed a statistically significant excess for lung cancer was the one that included taxi drivers and chauffeurs who worked multiple MER jobs for less than 10 years (OR = 2.5, 95% CI = 1.4, 4.8). For the same category the risk for individuals working in that job for more than 10 years was 1.2 (95% CI = 0.5, 2.6). A statistical significant positive trend

($p < 0.05$) with increasing employment of <2 years, 2 to 9 years, 10 to 19 years, and 20+ years was observed for truck drivers but not for other MER occupations. A statistically nonsignificant excess risk was also observed for heavy equipment operators, bus drivers, taxi drivers and chauffeurs, and mechanics employed for 10 years or more. All of the above-mentioned odds ratios were derived adjusted for birth cohort, usual daily cigarette use, and state of residence. Exposure to other occupational suspect lung carcinogens did not account for the excess risks detected.

Results of this large study provide evidence that workers in MER jobs are at an excess risk of lung cancer that is not explained by their smoking habits or exposures to other lung cancers. Because no information on type of engine had been collected, it was not possible to determine if the excess risk was due to exposure to diesel exhaust or gasoline exhaust or the mixture of the two. Among its limitations are possible bias due to misclassification of jobs reported by the large proportion of next-of-kin interviews and the problems in classifying individuals into uniform occupational groups based on the pooled data in the three studies that used different occupational classification schemes.

### 8.3.8 Steenland et al. (1990): A Case-Control Study of Lung Cancer and Truck Driving in the Teamsters Union

Steenland et al. conducted a case-control study of lung cancer deaths in the Teamsters Union to determine the risk of lung cancer among different occupations. Death certificates were obtained from the Teamsters Union files in the central states for 10,485 (98%) male decedents who had filed claims for pension benefits and who had died in 1982 and 1983. Individuals were required to have 20 years tenure in the union to be eligible to claim benefits. Cases comprised all deaths ($n = 1,288$) from lung cancer, coded as ICD 162 or 163 for underlying or contributory cause on the death certificate. The 1,452 controls comprised every sixth death from the entire file excluding deaths from lung cancer, bladder cancer, and motor vehicle accidents. Detailed information on work history and potential confounders such as smoking, diet, and asbestos exposure was obtained by questionnaire. Seventy-six percent of the interviews were provided by spouses and the remainder by some other next of kin. The response rate was 82% for cases and 80% for controls. Using these interview data and the 1980 census occupation and industry codes, subjects were classified

either as nonexposed, or as having held other jobs with potential diesel exhaust exposure. Data on job categories were missing for 12% of the study subjects. A second work history file was also created based on the Teamsters Union pension application that lists occupation, employer, and dates of employment. A three-digit U.S. census code for occupation and industry was assigned to each job for each individual. This Teamsters Union work history file did not have information on whether men drove diesel or gasoline trucks, and the four principal occupations were long-haul drivers, short-haul or city drivers, truck mechanics, and dock workers. Subjects were assigned the job category in which they had worked the longest.

The case-control analysis was done using unconditional logistic regression. Separate analyses were conducted for work histories from the Teamsters Union pension file and from next-of-kin interviews. Covariate data were obtained from next-of-kin interviews. Analyses were also performed for two time periods: employment after 1959 and employment after 1964. These two cut-off years reflect years of presumed dieselization; 1960 for most trucking companies and 1965 for independent driver and nontrucking firms. Data for analysis could be obtained for 994 cases and 1,085 controls using Teamsters Union work history and for 872 cases and 957 controls, using next-of-kin work history. When exposure was considered as a dichotomous variable, for both Teamsters Union and next-of-kin work history, no single job category had an elevated risk. From the next-of-kin data, diesel truck drivers had an odds ratio of 1.42 (95% CI = 0.74, 2.47) and diesel truck mechanics had an odds ratio of 1.35 (95% CI = 0.74, 2.47). Odds ratios by duration of employment as a categorical variable were then estimated. For the Teamsters Union work history data and when only employment after 1959 was considered, both long haul ($p < 0.04$) and short haul drivers (not significant) showed an increase in risk with increased years of exposure. The length of employment categories for which the trends were analyzed were 1 to 11 years, 12 to 17 years, and 18 years or more. Using 1964 as the cut-off date, long haul drivers continued to show a significant positive trend ($p = 0.04$) with an odds ratio of 1.64 (95% CI = 1.05, 2.57) for those who worked for 13+ years, the highest category. Short haul drivers, however, did not show a positive trend when 1964 was used as the cut-off date. Similar trend analysis was done for most next-of-kin data. A marginal increase in risk with increasing duration of employment as a truck driver ($p = 0.12$) was observed. For

truck drivers who primarily drove diesel trucks for 35 years or longer, the odds ratio for lung cancer was 1.89 (95% CI = 1.04, 3.42). The odds ratio for gasoline truck drivers was 1.34 (95% CI = 0.81, 2.22) and for truck mechanics was 1.09 (95% CI = 0.44, 2.66). No significant interactions between age and diesel exhaust exposure or smoking and diesel exhaust exposure were observed. All the odds ratios were adjusted for age, smoking, and asbestos in addition to various exposure categories.

The authors acknowledge several limitations of this study which include possible misclassifications of exposure and smoking habits, as information was provided by next of kin; lack of sufficient latency to observe lung cancer excess; and a small nonexposed group (n = 120). Also, concordance between Teamsters Union and next-of-kin job categories could not be easily evaluated because job categories were defined differently in each data set. No data were available on levels of diesel exposure for the different job categories. Given these limitations, the positive findings of this study are probably underestimated.

Table 8-2 summarizes the lung cancer case control studies.

## 8.4 CASE-CONTROL STUDIES OF BLADDER CANCER

### 8.4.1 Howe et al. (1980): Tobacco Use, Occupation, Coffee, Various Nutrients, and Bladder Cancer

This is a Canadian population-based case-control study conducted in the provinces of British Columbia, Newfoundland, and Nova Scotia. These areas were selected because they had cancer registries and were believed not to have concentrations of high-risk industries. All patients with newly diagnosed bladder cancer occurring in the three provinces between April 1974 and June 1976 were identified, and 77% of them were interviewed at home. A total of 480 male and 152 female case-control pairs were available for analysis. For each case one neighborhood control, matched by age (±5 years) and sex, was also interviewed at home to obtain data on smoking, occupation, dietary sources of nitrites and nitrates which convert to nitrosamines (nonpublic water supply and preserved meat products), and beverage consumption, including a detailed history of coffee consumption. A detailed smoking history was obtained. The occupational history included a chronological account of all jobs and the

## TABLE 8-2. EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF LUNG CANCER

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Williams et al. (1977) | 7,518 (3,539 males and 3,979 females) incident invasive cancers from the Third National Cancer Survey<br><br>Lung cancer cases:<br>32 in males<br>28 in females<br><br>Combined other cancer sites were used as controls. | Main lifetime, recent, and other employment information obtained at the time of survey<br><br>1970 Census Coding Scheme for Employment was used to code the occupations by one of the authors. | SNS elevated relative odds were observed among occupations of trucking, railroading, and mining. | Exposure estimation based on self-report that was not validated<br><br>47% Nonresponse<br><br>Control group consisted of other cancers probably diluting the risk estimation.<br><br>Small numbers in cause- specific cancers and individual occupations |
| Hall and Wynder (1984) | 502 Histologically confirmed lung cancers<br>Cases diagnosed 12 mo prior to interviews<br><br>502 Matched hospital controls without tobacco related diseases, matched for age, sex, race, and geographical area<br><br>Population from 18 hospitals in controls | Based on previous Industrial Hygiene Standards for a particular occupation, usual lifetime occupation coded as "probably high exposure" and "no exposure"<br><br>NIOSH standards used to classify exposures:<br>High<br>Moderate<br>Low | SNS excess risk after adjustment for smoking for lung cancer:<br>RR = 1.4 (1st criteria)<br>and<br>RR = 1.7 (NIOSH criteria) | Complete lifetime employment history not available<br><br>Self-reported occupation history not validated<br><br>No analysis by dose, latency, or duration of exposure<br><br>No information on nonoccupational diesel exposure |



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**TABLE 8-2 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF LUNG CANCER**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Damber and Larsson (1987) | 589 Lung cancer cases who had died prior to 1979 reported to Swedish registery between 1972 and 1977<br><br>582 Matched dead controls (sex, age, year of death, municipality) drawn from National Registry of Cause of Death<br><br>453 Matched living controls (sex, year of birth, municipality) drawn from National Population Registry | Occupations held for at least 1 year or more<br><br>Using a 5-digit code the occupations were classified according to Nordic Classification of Occupations | SS OR = 2.7 (≥1 year of employment)<br><br>SS OR = 9.8 (≥20 years of employment)<br><br>Adjustment for smoking was done.<br><br>SNS OR = 1.2 for professwional drivers (≥20 years of employment) with dead controls<br><br>SNS OR = 1.1 ≥20 years of employment) with living controls. | Uncertain diesel exhaust exposure<br><br>No validation of exposure done<br><br>Underground miners data not adjusted for other confounders such as radon, etc. |
| Lerchen et al. (1987) | 506 Lung cancer cases from New Mexico Tumor registry (333 males and 173 females)<br><br>Aged 25-84 years<br><br>Diagnosed between January 1, 1980, and December 31, 1982<br><br>771 (499 males and 272 females) frequency matched with cases, selected from telephone directory | Lifetime occupational history and selfreported exposure history was obtained.<br><br>Coded according to Standard Industrial Classification Scheme | No excess of relative odds was observed for diesel exhaust exposure. | Exposure based on occupational history and self report, which was not validated<br><br>50% Occupational history provided by next of kin<br><br>Absence of lung cancer association with asbestos suggests misclassification of exposure. |



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**TABLE 8-2 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF LUNG CANCER**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Garshick et al. (1987) | 1,319 Lung cancer cases who died between March 1, 1981, and February 28, 1982<br><br>2,385 Matched controls (two each, age and date of death)<br><br>Both cases and controls drawn from railroad worker cohort who had worked for 10 or more years | Personal exposure assessed for 39 job categories.<br><br>This was corrected with job titles to dichotomize the exposure into:<br>Exposed<br>Not exposed | SS OR = 1.41 (≤64 year age group<br><br>SS OR = 1.64 (≤64 year age group) for ≥20 years diesel exhaust exposure group when compared to 0- to 4-year exposure group<br><br>All ORs adjusted for life time smoking and asbestos exposure. | Probable misclassification of diesel exhaust exposure jobs<br><br>Years of exposure used as surrogate for dose<br><br>13% of death certificates not ascertained<br><br>Overestimation of smoking history |
| Benhamou et al. (1988) | 1,260 Histologically confirmed lung cancer cases<br><br>2,084 Non-tobacco-related disease matched controls (sex, age at diagnosis, hospital admission, and interviewer)<br><br>Occurring between 1976 and 1980 in France | Based on exposures determined by panel of experts<br><br>The occupations were recorded blindly using International Standard Classification of Occupations as chemical or physical exposures. | Significant excess risks were found in motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35) (smoking adjusted). | Exposure based on occupational histories not validated<br><br>Exposures classified as chemical and physical exposure, not specific to diesel exhaust |
| Hayes et al. (1989) | Pooled data from three different studies consisting of 2,291 male lung cancer cases<br><br>2,570 Controls | Occupational information from next of kin for all jobs held<br><br>Jobs classified with respect to potential exposure to known and suspected pulmonary carcinogens | SS OR = 1.5 for truck drivers (>10 years of employment)<br><br>SS positive trend with increasing employment as truck driver | Exposure data based on job description given by next of kin, which was not validated<br><br>Could have been mixed exposure to both diesel and gasoline exhausts<br><br>Job description could have lead to misclassification |



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**TABLE 8-2 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF LUNG CANCER**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Steenland et al. (1990) | 1,058 Male lung cancer deaths between 1982 and 1983<br><br>1,160 Every sixth death from entire mortality file sorted by social security number (excluding lung cancer, bladder cancer, and motor vehicle accidents)<br><br>Cases and controls were from Central State Teamsters who had filed claims (requiring 20-year tenure). | Longest job held: diesel truck driver, gasoline truck driver, both types of trucks, truck mechanic, and dock workers | As 1964 cutoff point:<br><br>SS OR = 1.64 for long haul drivers with 13+ years of employment<br><br>Positive trend test for long haul drivers (p = 0.04)<br><br>SS OR = 1.89 for diesel truck drivers of 35+ years of employment | Exposure based on job titles not validated<br><br>Possible misclassification of exposure and smoking, based on nextofkin information<br><br>Lack of sufficient latency |

Abbreviations:
OR = Odds ratio.
RR = Relative risk.
SNS = Statistically nonsignificant.
SS = Statistically significant.

number of years and months during which the respondent had worked in each job, experience in industries that were suspected a priori to increase the risk of bladder cancer, and exposure to any jobs that involved exposure to dust and fumes at the workplace. Relative risk estimates were computed using the linear logistic model applied to individually matched case-control pairs.

A base-line comparison of cases and controls showed that, whereas male patients were similar to controls on income and education, there was an excess of female cases with low family incomes and low levels of educational attainment. For both sexes the mean ages for cases and controls did not differ, and the times required for the interview were similar. An analysis by the a priori suspect industries showed elevated risks for a number of industries for males. These included the chemical (RR = 7.5, 95% CI = 1.7, 67.6), rubber (RR = 5.0, 95% CI = 0.6, 236.5), petroleum (RR = 5.3, 95% CI = 1.5, 28.6), medicine (RR = 2.6, 95% CI = 0.9, 9.3), and spray painting (RR = 1.8, 95% CI = 0.7, 4.6) industries. The excess risks were statistically significant only for the petroleum and chemical industries. The estimates did not change when the analysis was done separately for subjects who reported only one exposure and for those who reported exposure to more than one suspect industry. The estimates also remained unchanged after controlling for smoking. Too few females reported working in the a priori suspect industries to make any meaningful contribution to the analysis. Among males, statistically nonsignificant excess risks were observed for tanning, electric cable, photographic, commercial paint, tailoring, medicine, food processing, and agricultural industries. The analysis by exposure to dust and fumes in occupations other than those in the a priori suspect list detected the relative risks for diesel and traffic fumes (RR = 2.8, 95% CI = 0.8, 11.8). Statistically significant excess risks were observed for railroad workers (RR = 9.0, 95% CI = 1.2, 394.5) and welders (RR = 2.8, 95% CI = 1.1, 8.8). For occupations other than those on the a priori list for males and females, statistically significant excesses were detected for metal machinists (RR = 2.7, 95% CI = 1.1, 7.6), metal recorders (RR = 2.6, 95% CI = 1.0, 7.3), and nursery men (RR = 5.5, 95% CI = 1.2, 51.1). Statistically nonsignificant excesses were also detected for exposure to two chemicals: benzidine and its salts, RR = 1.3, and *bis*-chloromethyl ether, RR = 5.0. A detailed analysis was done for cigarette smoking, which demonstrated statistically significant increasing bladder cancer risk with increasing

duration of smoking, total lifetime consumption of packs of cigarettes, and average frequency of cigarettes per day. In males the highest significant risk was observed for latency of less than 35 years; after that time the risk reduced slightly with increasing latency. In females the highest significant risk was for more than 35 years of latency. Risks were elevated for males consuming all types of coffee and for females consuming instant coffee. Hair dye usage in females and phenacetin usage in males and females carried no risk. Significant risks for use of artificial sweeteners and use of nonpublic water supplies (nitrates and nitrites) were found among males only.

This study was mainly designed to evaluate the various risk factors for bladder cancer such as smoking, coffee consumption, nitrates and nitrites in diet, etc. The major limitation of this study, as the authors noted, was that the three selected provinces did not have high concentrations of industries suspected to be linked to bladder cancer. An excess risk was, however, detected for railroad workers and for those in the "exposed to diesel and traffic fumes category." Risks for those exposed to "diesel fumes only" were not available nor do we know the exact job title of the railroad workers and the type of engines they were operating. The authors also did not detail the method by which they coded the information given by respondents in response to questions on exposure to dust and fumes into the various categories they used in the analysis. These analyses were done for subjects who reported having "ever been exposed" versus "never been exposed" to these fumes, and, although detailed chronological work histories were obtained, no attempt was made to develop a lifetime cumulative exposure index to diesel fumes. In multiple logistic regression models, the authors used the a priori high-risk occupations; hence, nothing can be concluded about exposure to diesel exhaust for occupations that were not part of that list. The authors provided no explanation on possible selection bias as only 77% of the eligible population was included in the study.

### 8.4.2 Wynder et al. (1985): A Case-Control Study of Diesel Exhaust Exposure and Bladder Cancer

A case-control study of diesel exhaust exposure and bladder cancer risk was conducted by Wynder et al. (1985). Cases and controls were obtained from 18 hospitals located in six U.S. cities between January 1981 and May 1983. Cases were individuals with histologically

confirmed primary cancer of the bladder, diagnosed within 12 mo prior to the interview. Controls were individuals with nontobacco-related diseases who were matched to the cases by age (within 8 years), race, year of interview, and hospital of admission. Women were excluded from the study since the focus was on male-dominated occupations. A structured questionnaire was administered in the hospital to cases and controls to elicit information on usual occupation, smoking history, alcohol and coffee consumption, as well as other demographic factors.

Two methods were used to define occupational exposure to diesel exhaust. First, occupational titles defined by the industrial hygiene standards as probable high exposure were classified as exposed or not exposed to diesel exhaust. The probable high exposure category consisted of bus and truck drivers, heavy equipment operators and repairmen, railroad workers, and warehousemen. In the second method, guidelines set by NIOSH were used to classify occupations based on exposure to diesel exhaust. In this method, the estimated proportion of exposed workers was computed for each occupational category by using the NIOSH estimates of the exposed population as the numerator and the estimates of individuals employed in each occupational category from the 1970 census as the denominator. Occupations estimated to have at least 20% of their employees exposed to diesel exhaust were defined as "high exposure," those with 10 to 19% of their employees exposed as "moderate exposure," and those with less than 10% of their empolyees exposed as "low exposure." The odds ratio was used as a measure of association to assess the relationship between bladder cancer and diesel exhaust exposure. The overall participation among those eligible and available for interview was 75 and 72% in cases and controls, respectively.

A total of 194 bladder cancer cases and 582 controls were examined, and the two groups were found to be comparable by age and education. Except for railroad workers who had relative odds of 2.0 based on two cases and three controls (95% CI = 0.34, 11.61), the relative odds were less than one for other diesel exhaust exposure occupations such as bus and truck drivers, warehousemen, material handlers, and heavy equipment workers. When the risk was examined using the NIOSH criteria for high, moderate, and low exposure, relative odds were 1.68 and 0.16 for high and moderate, respectively, with low as the referent group; neither was statistically significant. Cases and controls were compared by smoking status. Cases were more likely to be current cigarette smokers than controls.

Current smokers of 1 to 20 cigarettes/day had relative odds of 3.64 (95% CI = 2.04, 6.49), current smokers of 21+ cigarettes/day had relative odds of 3.51 (95% CI = 2.00, 6.19), while exsmokers had relative odds of 1.72 (95% CI = 1.01, 2.92). After controlling for smoking, there was no significant increase in the risk of bladder cancer for occupations with diesel exhaust exposure compared to occupations without diesel exhaust exposure. A synergistic effect between the two also was not detected.

This study has two major methodologic limitations, both pertaining to exposure classification. First, the use of "usual" occupation may have lead to misclassification of those individuals who had held a previous job with diesel exhaust exposure that was not their usual occupation; this may have resulted in reduced power to detect weak associations. Second, since there was no information on amount or duration of diesel exhaust exposure, no analysis of dose-response relationships could be done. Also, no information was available on other confounding risk factors of bladder cancer such as urinary retention, amphetamine abuse, and smoking within the confined space of a truck cab, all of which are life-style factors specific to the truck driving occupation.

### 8.4.3 Hoar and Hoover (1985): Truck Driving and Bladder Cancer Mortality in Rural New England

This study investigated the relationship between the occupation of truck driving and bladder cancer mortality in a case-control study in New Hampshire and Vermont. Cases included all white residents of New Hampshire and Vermont who died from bladder cancer (eighth revision of the ICD) between 1975 and 1979. Death certificates were provided by the vital records and health statistics office of the two states, and the next of kin were traced and interviewed in person. Two types of controls were selected for each case. One control was randomly selected from all other deaths, excluding suicides, and matched on state, sex, race, age (±2 years), and year of death. The second control was selected with the additional matching criteria of county of residence. Completed interviews were obtained from 325 (out of 410) next of kin for cases and 673 (out of 923) controls. Information on demographic characteristics, lifetime occupational and residential histories, tobacco use, diet, and medical history were obtained on each subject. The odds ratio was calculated to ascertain a measure of association between truck driving and bladder cancer. Because separate analyses of the

two control series gave similar results, the two control series were combined. Also, because matched analyses yielded results similar to those provided by the unmatched analyses, results of the unmatched analyses were presented.

Sixteen percent (35) of the cases and 12% (53) of the controls had been employed as truck drivers yielding an odds ratio of 1.5 (95% CI = 0.9, 2.6) after adjustment for county of residence and age at death. For New Hampshire, the odds ratio was 1.3 (95% CI = 0.7, 2.3), and for Vermont the odds ratio was 1.7 (95% CI = 0.8, 3.4). For a large number of subjects, the next of kin were unable to give the durations of truck driving, and there was an inconsistent positive association with years of truck driving. Crude relative odds were not altered after adjustment for coffee drinking, cigarette smoking, and education as a surrogate for social class. Little variation in risks was seen when the data were analyzed by the industry in which the men had driven trucks. No relationship was seen between age at which employment as a truck driver started and occurrence of bladder cancer. Analysis by duration of employment as a truck driver and bladder cancer showed a positive trend of increasing relative odds with increasing duration of employment. The trend test was statisically significant ($p = 0.006$). The odds ratio was statistically significant for the 5- to 9-years employment category only (OR = 2.9, 95% CI = 1.2, 6.7). Similarly, analysis by calendar year first employed showed a statistically significant odds ratio for 1930 to 1949 (OR = 2.6, 95% CI = 1.3, 5.1), whereas relative odds were not significant if they were employed prior to 1929 or after 1950.

The effects of reported diesel exhaust exposure from fuel or engines in truck driving or other occupations were then analyzed. An odds ratio of 1.8 (95% CI = 0.5, 7.0) was derived for those who were exposed to diesel exhaust during their truck driving jobs as compared to an odds ratio of 1.5 (95% CI = 0.8, 2.7) for those not reporting diesel exhaust exposure. Analysis by duration of exposure (0, 1 to 19 years, 20 to 29 years, 30 to 39 years, and 40+ years) to diesel fuel or engines in other occupations, which were "self-reported" by participants, showed a statistically significant positive trend ($p = 0.024$) for bladder cancer, although none of the individual odds ratios in these duration categories were statistically significant.

This study investigated an association between truck driving and bladder cancer in an attempt to understand the reasons for the high rates of bladder cancer in rural areas of

New Hampshire and Vermont. Although an elevated odds ratio for bladder cancer (not statistically significant) was observed for reported truck-driving occupations, there was insufficient evidence to conclude that the excess risk of bladder cancer was due to exposure to diesel emissions. This is because the excess bladder cancer risk was observed for all truck drivers irrespective of their exposure to diesel emissions. Also, no information was provided on the confounding effects of other aspects of the road environment such as urinary retention, amphetamine abuse, and concentrated cigarette smoke within the truck cab. Other limitations of this study include the use of next of kin for occupational histories who may either under- or overreport occupations and the use of death certificates with their inherent problems of misclassification.

### 8.4.4 Steenland et al. (1987): A Case-Control Study of Bladder Cancer Using City Directories as a Source of Occupational Data

The primary objective of the study was to test the usefulness of city directories as a source of occupational data in epidemiologic studies of illness and occupational exposure. Commercial city directories provide data on occupations and employers and are compiled from a door-to-door yearly census of all residents 18 years old and older. The directories are available in most medium-size cities in the United States. A unique feature of city directory data is that they identify specific employers, and as the authors suggest, this information may be better than death certificates for rapid, inexpensive, record-based, epidemiologic studies.

A case-control study was conducted of male bladder cancer deaths in Hamilton County (including Cincinnati), OH. This county was selected because it is in an industrialized area with high bladder cancer rates and also because city directories cover approximately 85% of the people living in the county. A computerized list of all male bladder cancer deaths (n = 731) and all other male deaths (n = 95,057), with the exclusion of deaths from urinary tract tumors and pneumonia, that occurred between 1960 and 1982 was obtained from the Ohio Department of Vital Statistics. Death certificates had been coded by a nosologist according to the ICD code in use at the time of death. A pool of six controls was created for each case matched on sex, residence in Hamilton County at time of death, year of death, age at death ($\pm$5 years), and race.

Two types of analysis were performed, one based on city directory data and the other based on death certificate data. In the former, cases and controls were restricted to individuals who had at least one yearly directory listing with some occupational data. The first two controls from the pool of six who met the requirements were selected. This analysis involved 648 cases (627 cases had 2 controls and 21 cases had only 1 control) and 1,275 controls.

The death certificate analysis involved all 731 cancer deaths, with two controls per case. In most cases, the same two controls were used in this analysis too. The usual lifetime occupation and industry on the death certificate was abstracted from them. Data on occupation and industry were coded with a three-digit U.S. census code using the method adopted by the U.S. Bureau of the Census. Five of the occupational data were recorded for occupation and industry by a second coder, with a high degree of reproducibility. Odds ratios were calculated for bladder cancer using a Mantel-Haenszel procedure.

The city directory data identified four employers significantly associated with bladder cancer deaths; only one of them was identified by the death certificate data that provided only lifetime type of industry rather than the name of a specific employer. The industries represented by the four employers were a chemical plant, printing company, valve company, and machinery plant. The city directory data analysis demonstrated significant positive associations for quite a few occupations. The occupations that had at least 10 cases or more were engineers ($OR = 3.00$, $p = 0.01$), carpenters ($OR = 2.36$, $p < 0.01$), tailors ($OR = 2.56$, $p < 0.01$), and furnace operators ($OR = 2.5$, $p = 0.03$). Relative odds were increased significantly with increased duration of employment ($\geq 20$ years) for truck drivers ($OR = 12$, $p = 0.01$) and furnace operators (based on four cases and no controls, $p = 0.05$). For occupations in which subjects had ever been employed, a significant increase in the relative odds with increased duration of employment was observed for the railroad industry ($\geq 20$ years of employment, $OR = 2.21$, $p < 0.05$). Both truck driving and railroad industry occupations involve diesel emission exposures.

The analysis of death certificate data yielded associations in the same direction for most of the occupations. A check of the validity of city directory data indicated that 77% of the listings agreed with the social security earnings report for the employer in any given year. A comparison of city directory and death certificate information on occupations indicated a

match for occupation between at least one city directory listing and occupation on death certificates for 68.3% of the study subjects.

This study demonstrated that city directories are a relatively inexpensive and accessible source of occupational data for epidemiologic studies. Limitations of this study include the problem in tracing women because of the change from maiden to married name and the availability of data for only the year of residence in the city. They are superior to death certificates in being able to identify high-risk employers in specific geographic sites. Although death certificate data reflect usual lifetime occupation, city directories yield data on short-term jobs, some of which may involve critical exposure. Thus, a combination of the two approaches may be most productive in record-based hypothesis-generating studies. The occupational data were missing for 15%, whereas employer data were missing for 36% in the city directory. In the context of the mentioned pros and cons of using city directories, this study found an excess risk for bladder cancer among two occupations with potential diesel exposure: (1) truck drivers and (2) railroad workers. Two sources of bias that may have influenced these findings are selection bias arising from the use of deaths instead of incident cases, because survival for bladder cancer is high, and the absence of data on confounding factors such as smoking, beverage consumption, and medication use.

### 8.4.5 Iscovich et al. (1987): Tobacco Smoking, Occupational Exposure, and Bladder Cancer in Argentina

This is a hospital-based case-control study of bladder cancer conducted in La Plata, Argentina, to estimate the risk of bladder cancer associated with different types of tobacco, beverages, and occupational exposures. Bladder cancer is one of the most common cancers among males in the La Plata area.

Cases were selected from patients with a histologically confirmed diagnosis of bladder cancer (transitional, squamous-cell, or nonspecific cell type) admitted to the 10 general hospitals in the greater La Plata area (population in 1980 = 580,000) between March 1983 and December 1985. Cases with true bladder papilloma and individuals who were residents of greater La Plata for less than 5 years were excluded. Of the 120 cases eligible to participate, 1 died prior to the interview, 2 refused to participate, and the remaining 117 cases, representing 60% of the incident cases registered in the registry, were

interviewed. Two control groups (117 neighborhood and 117 hospital controls) were matched by sex and age (±5 years). Of the 117 cases, 99 were males and 18 were females. Hospital controls, selected from the same hospital as the cases, were hospitalized for the first time within 3 mo of diagnosis of the illness of the cases. Twelve percent of the hospital controls had illnesses known to be associated with tobacco smoking. Neighborhood controls were sampled from among persons living in the same block. The interviewer proceeded north in the block where the case resided and selected the first control who met the matching criteria. Seven hospital controls could not be interviewed because of their poor physical health and were replaced. Three neighborhood controls refused to participate and were replaced. Cases and hospital controls were interviewed at the hospital and the neighborhood controls at their homes to collect data on demographic, socioeconomic, and medical variables, detailed smoking habits, and alcoholic and other beverages consumed.

The interviews were done by trained interviewers, two physicians, and a social worker. The cases and hospital controls were interviewed in the hospital by the physicians; hence, the interviews could not be conducted "blind". A detailed occupational history was obtained for the three occupations of longest duration and the most recent one. For each job title, the activity of the plant and type of production was also ascertained. Job titles were coded according to the International Labor Union (ILO) 1970 classification. Plant activity and type of production were coded according to the United Nations 1975 classification categories. Information was also collected on exposure to 33 chemical and physical agents, which included confirmed or suspected bladder carcinogens. A detailed history of smoking habits was also obtained, and individuals were categorized as current smokers if they were smoking at present or if they had stopped smoking less than 2 years previously. Exsmokers were those who ceased smoking at least for 2 years or more than 2 years previously. For each subject a cumulative lifelong consumption of cigarettes by type was estimated, and an average consumption of cigarettes/day was computed.

Relative risks were computed for occupational factors using the unconditional logistic regression method, adjusting for age and tobacco smoking. These risks were derived for those who were ever employed in that occupation versus those who were never employed in that occupation. Socioeconomic status of cases and neighborhood controls was similar but there were fewer professionals and more manual workers among hospital controls compared

with cases. Occupational variables included job title and type of activity of the plant. Significant excess risks were observed for truck and railroad drivers (RR = 4.31, $p < 0.002$) and oil refinery workers (RR = 6.22, $p < 0.02$). The risk for truck and railroad drivers was reduced after adjusting for smoking, whereas that for oil refinery workers increased after adjusting for smoking (no RRs were presented). The adjusted relative risks were not reported. A positive but nonsignificant association was observed for printers (RR = 2.6, $p < 0.77$).

This study identified smoking and coffee drinking as the major risk factors for bladder cancer in this area. The overall age-adjusted relative risk in males for current smokers relative to nonsmokers was 7.2 (95% CI = 3.0, 20.1) with dose-response relationships observed for the average daily amount as well as for duration of smoking. A strong dose-response relationship was also observed for coffee drinking in males with a relative risk of 12 (95% CI = 4.3, 33.31) for those drinking more than three cups of coffee per day after adjusting for the effect of smoking. No association was found with use of saccharin in males. No results were presented for females for these risk factors.

This case-control study was conducted primarily to determine the reasons for the high rates of bladder cancer in the La Plata region of Argentina. Only 60% of the cases registered in the cancer registry were interviewed, and no information was provided for the remaining 40% eligible nonrespondents to determine if the study sample was selectively biased in any way. The sample size of 117 was small, and the analysis of males reduced it to 99. Although the use of two different types of control groups is a strength of this study, none of the interviews were done blind, and it appears that the hospital interviews were done by the physicians and the neighborhood interviews were done by the social worker. Job titles were used as surrogates of exposure, but the authors state that although they did attempt to analyze by an exposure index derived from a job exposure matrix (details not provided), they found no difference in exposure between cases and controls. This explanation is ambiguous. The authors also grouped truck and railroad drivers together for reasons not mentioned, and did not present separate risk estimates. A table showing the distribution of cases and controls for selected activities or professions did not indicate if the data pertain to both sexes or males only, and the text did not clarify that either. The reported significant excess risks for truck and railroad drivers were reduced after adjusting for smoking, but it

was not known if the statistical significance persisted. No analysis was provided for the data collected in the interviews on exposures to the 33 chemical and physical agents, and it was not known if the truck and railroad drivers were operating diesel engines. Although rare in the La Plata area, the authors acknowledge the occupations known to be associated with bladder cancer (dye, rubber, leather, and textile workers).

### 8.4.6 Iyer et al. (1990): Diesel Exhaust Exposure and Bladder Cancer Risk

This study is a hospital-based case-control study of bladder cancer and potential exposure to diesel exhaust using data from a large ongoing case-control study of tobacco-related neoplasms conducted by the American Health Foundation. An earlier study by Wynder et al. (1985) looked at the relationship between occupational exposure to diesel exhaust and the risk of bladder cancer. For this study, the objective was to evaluate the relationship between the different measures of exposure to diesel exhaust, occupational and self-reported, and the risk of bladder cancer. Cases comprised 136 patients with histologically confirmed primary cancer of the urinary bladder interviewed at 18 hospitals in six U.S. cities. Two controls were selected per case matched for sex, age, (within 2 years), race, hospital, and year of interview. A total of 160 controls had nontobacco related malignancies distributed as follows: stomach cancer (6%), colorectal cancer (20%), prostate cancer (6%) and leukemia or lymphoma (8%). Among the 112 controls with nonmalignant diseases, 3% had benign neoplasms, 6% had hyperplasis of the prostate, and 6% had dorsopathies. Distribution of the other nonmalignant illnesses was not provided. Occupational history included information on usual occupation and up to five other jobs. Exposure to diesel exhaust in hobby activities also was collected. For the purpose of this analysis, occupations were aggregated a priori into three categories: low probability of exposure (reference group), possible exposure, and probable exposure. Analyses were also done for self-reported exposure to diesel exhaust. Risk estimates were obtained by unconditional logistic regression using PROC LOGIST of SAS. Cases and controls were first compared by age, race, education, and smoking habit. Cases were found to be less educated than controls ($p < 0.05$). Crude odds ratios for diesel exhaust exposure, based on occupational or self-reported exposure, were not significantly elevated after controlling for

smoking and educational status (OR = 1.2, 95% CI = 0.8, 2.0). When individual occupations were analyzed separately, truck drivers showed no excess risk (OR = 0.48, 95% CI = 0.15, 1.56).

The authors concluded that their study does not support the hypothesis of an association between exposure to diesel exhaust and bladder cancer. Several significant limitations of exposure assessment and analysis are evident in this study. In the introduction, the authors stated that they refined the definition of exposure to diesel exhaust by obtaining a lifetime occupational history, but in the methods section they stated that they restricted analysis to usual occupational history and five other jobs, which was not that different from their earlier study (Wynder et al., 1985). The terms, low probability of exposure, possible exposure, and probable exposure also were not clearly defined. Information on duration of employment or exposure was not presented, and no attempts were made to validate occupational history. No information was available on calendar years of employment in the truck-driving industry or the locomotive occupations. Because diesel trucks and locomotives were introduced in the mid 1950s and the dieselization was completed by 1960, it would be important to use 1960 as a cut-off date and to restrict analysis to subjects who worked in these industries after that date. No information on nonrespondent cases and controls was provided. The authors indicated in the method section that cases were individually matched to controls, but they performed an unmatched analysis to calculate the odds ratios and do not address why they did not preserve the matching in the analysis, especially because such an analysis could bias the risk estimates to unity.

### 8.4.7 Steineck et al. (1990): Increased Risk of Urothelial Cancer in Stockholm from 1985 to 1987, After Exposure to Benzene and Exhausts

This study was conducted to investigate the association between benzene, diesel, and petrol exhausts as well as some other industry-related chemicals and the risk of urothelial cancer. Designed as a population-based case-control study, it was conducted among all men born between 1911 and 1945 and living in the County of Stockholm for all or part of the observation period (September 15, 1985, to November 30, 1987). All incident cases of urothelial cancer and squamous-cell carcinoma of the lower urinary tract were contacted for inclusion in the study. Controls were selected by stratified random sampling during the

observation period from a computerized register for the population of Stockholm. A postal questionnaire was sent to study subjects at their homes to collect information on occupational history. The questions on occupation included exposure to certain specified occupations/industries/chemicals and lists of all jobs held and duration in each job. An industrial hygienist, unaware of case-control status, classified subjects as being exposed or unexposed to 38 agents and groups of substances including 17 exposure categories with aromatic amines. Using all the exposure information, the exposure period was defined and the annual dose was rated as low, moderate, or high based on the accumulated dose (exposure duration multiplied by intensity of exposure) during the course of 1 average year for the defined exposure period. Swedish and international data were used to classify subjects as exposed, based on air concentrations in the work environment that were higher than for the general public, or skin contact with liquids of low volatility. To allow for latency, the authors ignored exposures after 1981. Data were gathered from 256 cases and 287 controls. Controls were selected by stratified random sampling four times from the computerized register during the observation period of the population of the County of Stockholm. These subjects comprised 80% of eligible cases and 79% of eligible controls. Nine of the cases and 16% of the controls refused to participate in the study. The distribution of urothelial cancers was as follows: 5 tumors in the renal pelvis, 243 in the urinary bladder, 5 in the ureter, none in the urethra, and 3 at multiple sites. Two cases who were exposed to a high annual dose of aromatic amines were omitted from all further analysis to eliminate their confounding effects. Crude relative risks were calculated for men classified as exposed or not exposed to several substances. Twenty-five cases and 19 controls reported having been exposed to diesel exhaust, yielding an odds ratio of 1.7 (95% CI = 0.9, 3.3). The corresponding relative odds for petrol exhausts based on 24 cases and 24 controls were 1.0 (95% CI = 0.5, 1.9). Odds ratios were then calculated for low, moderate, and high levels of the annual dose adjusted for smoking and year of birth. For diesel exhausts, the odds ratio for low levels was 1.3 (95% CI = 0.6, 3.1), for moderate levels was 2.2 (95% CI = 0.7, 6.6), and for high levels was 2.9 (95% CI = 0.3, 30.0) indicating a dose response. The corresponding odds ratios for petrol exhausts were 0.6 (95% CI = 0.3, 1.3), 1.4 (95% CI = 0.5, 3.7), and 3.9 (95% CI = 0.4, 35.5). Restricting the analysis to only moderate or high annual doses of exposure adjusted for year

of birth and smoking showed a sevenfold increased risk for subjects exposed to both diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8). For exposure to diesel (OR = 1.1) and petrol (OR = 1.0) exhausts alone, no excess risk was detected in this analysis. Odds ratios were calculated for low, moderate, and high exposure to benzene with rates of 1.7 (95% CI = 0.6, 5.1) for low annual doses, 1.1 (95% CI = 0.3, 4.5) for moderate annual doses, and 3.0 (95% CI = 1.0, 8.7) for high annual doses.

The authors discuss misclassification and confounding as sources of bias in this study. To examine misclassification they compared hygienist-assessed exposure and self-reported exposure for printing ink and found a higher relative risk and fewer exposed subjects for hygienist-assessed exposure indicating that specificity was a problem for self-reported exposure. It is not known to what extent this may have affected the risk estimates for diesel exhausts since data on self-reported exposure to diesel are not presented. They also mention the possibility of exposure misclassification from using an average annual dose in which a person exposed to an agent at a high level for a few working days and a person exposed to a low level for many days are both rated as exposed to low annual doses. Although statistically nonsignificant elevated odds ratios of 1.3, 2.3, and 2.9 were derived for low, moderate, and high levels of diesel exposure, the authors state that some of their subjects may have later worked in jobs with benzene exposure, and because an elevated risk was detected for benzene exposure, this confounding effect may explain some of the excess risk. An almost statistically significant interaction was observed for exposure to combined diesel and petrol exhausts (OR = 7.1, 95% CI = 0.9, 58.8), which changed to 5.1 (95% CI = 0.6, 43.3) after adjustment for benzene exposure, again providing evidence for the confounding role of benzene exposure in explaining some of the observed results.

Table 8-3 summarizes the bladder cancer case-control studies.

## 8.5 DISCUSSION AND SUMMARY

Certain extracts of diesel exhaust have been demonstrated to be both mutagenic and carcinogenic in animals and in humans. Animal data are suggestive that diesel exhaust is a pulmonary carcinogen among rodents exposed by inhalation to high doses over long periods

## TABLE 8-3. EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF BLADDER CANCER

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Howe et al. (1980) | 480 Male case-control pairs<br><br>152 Female case-control pairs<br><br>Cases diagnosed between April 1974 and June 1976 in three Canadian provinces<br><br>Matched on age and sex | Based on occupational history of jobs involving exposure to dust and fumes<br><br>A priori suspect industries | SNS RR = 2.8 for diesel and traffic fumes<br>SS RR = 9.00 for railroad workers | Exposure based on occupational history, which was not validated<br><br>Diesel exhaust and traffic fumes were combined.<br><br>Only 77% of eligible population included in the study |
| Wynder et al. (1985) | 194 Histologically confirmed male cases between the ages of 20-80 years<br><br>582 Matched controls (age, race, year of interview, and hospital of admission); diseases not related to tobacco use<br><br>From 18 hospitals located in six U.S. cities between January 1981 and May 1983 | Occupational titles were defined by Industrial Hygiene Standard into dichotomous "exposed" and "not exposed".<br><br>Also defined by NIOSH standards into "high exposure", "moderate exposure", and "low exposure" | SNS ORs were 1.68 and 0.16 for high and moderate exposure, respectively, as compared to low exposure. | Exposure based on usual occupation may have lead to misclassification.<br><br>Dichotomous classification made dose-response analysis unattainable.<br><br>No data on other confounders such as smoking |



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**TABLE 8-3 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF BLADDER CANCER**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Hoar and Hoover (1985) | Population-based, case-control study<br><br>325 Cases from the residents of New Hampshire and Vermont who died of bladder cancer between 1975 and 1979<br><br>A total of 673 controls were chosen from other deaths during the same time period.<br><br>Two matched controls (age, sex, race, state, year of death, and second one matched also on county of residence) | Lifetime occupational history obtained from next of kin | SS OR = 2.9 for 5 to 9 years of employment as truck driver but not for ≥10 years of employment<br><br>Positive trend ($p = 0.006$) observed with increasing duration of employment as truck driver | Exposure defined as occupation of "truck driver" (i.e., it could have been diesel or gasoline or both)<br><br>No histogical confirmation of bladder cancer diagnosis<br><br>No data on other confounders such as other exposures, smoking, etc. |
| Steenland et al. (1987) | 648 Male bladder cancer deaths from Hamilton County, OH<br><br>1,275 Matched controls from other deaths (pool of six controls for each case, excluding urinary tract tumors and pneumonias matched on sex, age at death, year of death, race) | Occupation or industry listed in city directory and on death certificates. | OR = 12 ($p = 0.01$) for truck drivers with ≥20 years of employment<br><br>OR = 2.21 ($p \leq 0.05$) for railroad workers with >20 years of employment | Exposure based on city directory or death certificate listing that was not validated.<br><br>Lack of controlling for confounders<br><br>City directory usually has short-term job listing<br><br>Missing data on 15% of occupations and 36% for employers in the directory |



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**TABLE 8-3 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF BLADDER CANCER**

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Iscovich et al. (1987) | 117 Histologically confirmed bladder cancer cases (60% of all incident cases)<br><br>117 Hospital controls and 117 neighborhood controls (matched on age and sex)<br><br>Cses and hospital controls from 10 general hospitals in greater La Plata between March 1983 and December 1985 | Past and present occupational data were collected by questionnaire.<br><br>An exposure index based on a job exposure matrix was generated. | SS OR = 4.3 for truck and railway drivers<br><br>SS RR = 6.2 for oil refinery workers | Exposure based on job held that was not validated<br><br>40% of eligible cases were nonrespondent.<br><br>Small sample size<br><br>Interviewers were not "blind" to the status of an individual, and this could have biased the findings.<br><br>Truck and railroad drivers were grouped together.<br><br>Not adjusted for other confounding exposures such as dye, rubber, etc. |
| Iyer et al. (1990) | 136 histologically confirmed bladder cancer cases<br><br>272 controls, two each matched on sex, age, race, hospital, and year of interview (160 malignant, 112 nonmalignant)<br><br>From 18 hospitals in six U.S. cities | Lifetime occupational history<br><br>Self-reported diesel exhaust exposure<br><br>Exposure aggregated a priori into:<br>Low probability<br>Possible<br>Probable | No excess found. | Exposure based on self report, which was not validated<br><br>Although lifetime occupational history was obtained, analysis was restricted to usual occupation.<br><br>A priori classification was ambiguous. |



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TABLE 8-3 (cont'd). EPIDEMIOLOGIC STUDIES OF THE HEALTH EFFECTS OF EXPOSURE TO DIESEL EXHAUST: CASE-CONTROL STUDIES OF BLADDER CANCER

| Authors | Population Studied | Diesel Exhaust Exposure Assessment | Results | Limitations |
|---|---|---|---|---|
| Steineck et al. (1990) | Population based study from County of Stockholm<br><br>Men born between 1911 and 1945<br><br>256 (243 bladder) urinary tract cancer incident cases (80% of eligibles)<br><br>287 Controls (79% of eligibles) from population of Stockholm<br><br>Observation period September 15, 1985, to November 30, 1987 | Occupational history classified into exposed and nonexposed by industrial hygienist "blind" towards case or control status<br><br>Using all exposure information, annual dose rated as "low", "moderate", and "high" | SNS OR = 1.3 for low, OR = 2.2 for moderate, and OR = 2.9 for high exposure were observed for diesel exposure.<br><br>SNS OR = 7.1 observed for diesel and gasoline exhaust combined exposure | Elaborate exposure history classification not used to advantage by simultaneous adjustment<br><br>Misclassification in exposure may have occurred<br><br>Small sample size of only 25 cases and 19 controls were exposed to diesel exhaust.<br><br>Confounding by other exposures not accounted for, except benzene |

Abbreviations:
OR = Odds ratio.
RR = Relative risks.
SNS = Statistically nonsignificant.
SS = Statistically significant.



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of time. Because large working populations are currently exposed to diesel exhaust and because nonoccupational ambient exposures currently are of concern as well, the possibility that exposure to this complex mixture may be carcinogenic to humans has become an important public health issue.

Because diesel emissions become diluted in the ambient air, it is difficult to study the health effects in the general population. Nonoccupational exposure to diesel exhaust is worldwide in urban areas. Thus, "unexposed" reference populations used in occupational cohort studies are likely to contain a substantial number of individuals who are nonoccupationally exposed to diesel exhaust. Furthermore, the "exposed" group in these studies is based on job titles which in most instances are not verified or correlated with environmental hygiene measurement. The issue of health effect measurement gets further complicated by the fact that occupational cohorts tend to be healthy and have below-average mortality, usually referred as the "healthy worker effect". Hence, the usual standard mortality ratios observed in cohort mortality studies are underestimations of real risk.

A major difficulty with the occupational studies considered here was the measurement of the actual diesel exhaust exposure. Because all the cohort mortality studies were retrospective, the assessment of health effects from exposure to diesel exhaust was naturally indirect. In these occupational settings, no systematic quantitative records of ambient air were available. Most studies compared men in job categories with presumably some exposure to diesel exhaust with either standard populations (presumably no exposure to diesel exhaust) or with men in other job categories from industries with little or no potential for diesel exhaust exposure. A few studies have included measurements of diesel fumes, but there is no standard method for the measurement. No attempt is made to correlate these exposures with the cancers observed in any of these studies, nor is it clear exactly which extract should have been measured to assess the occupational exposure to diesel exhaust. All studies have relied on the job categories or self report of exposure to diesel exhaust. In the studies by Garshick et al. (1987, 1988), the diesel exhaust-exposed job categories were verified based on an industrial hygiene survey done by Woskie et al. (1988a,b). It was found by the investigators that in most cases the job titles were good surrogates for diesel exhaust exposure. Also, in this railroad industry where only persons who had at least 10 years of work experience were included in the study, the workers tended not to change

job categories over the years. Thus, a job known only at one point in time was a reasonable marker of past diesel exhaust exposure. Unfortunately the exposure was only qualitatively verified. The quantitative use of this information would have been much more meaningful. The occupations involving potential exposure to diesel exhaust are miners, truck drivers, transportation workers, railroad workers, and heavy equipment operators.

With the exception of the study by Waxweiler et al. (1973), there have been no known studies of miners to assess whether diesel exhaust is associated with lung cancer. Currently, there are about 385 underground metal mines in the United States. Of these, 250 have been permanently operating, whereas 135 have been intermittently operating (Steenland, 1986). Approximately 20,000 miners are employed, but all of them are not currently working in the mines. Diesel engines were introduced in the metal mines in the early to mid 1960s. Although all these mines use diesel equipment, it is difficult to estimate how many of these miners were actually exposed to diesel fumes.

Diesel engines were introduced in coal mines at an even later date, and their use is still quite limited. In 1983, approximately 1,000 diesel units were in place in underground coal mines, up from about 200 units in 1977 (Daniel, 1984). The number of units per mine varies greatly; one mine may account for over 100 units.

Even if it were possible to estimate how many miners (metal and coal) were exposed to diesel exhaust, it would be very difficult to separate out the confounding effects of other potential pulmonary carcinogens, such as radon decay products, heavy metals (such as arsenic, chromium), etc. Furthermore, the relatively short latency period limits the usefulness of these cohorts of miners.

### 8.5.1 The Cohort Mortality Studies

The cohort studies mainly demonstrated an increase in lung cancer. Studies of bus company workers by Waller (1981), Rushton et al. (1983), and Edling et al. (1987) failed to demonstrate any statistically significant excess risk of lung cancer, but these studies have certain methodological problems, such as small sample sizes, short follow-up periods (just 6 years in the Rushton et al. study), lack of information on confounding variables, and lack of analysis by duration of exposure, duration of employment, or latency, that preclude their use in determining the carcinogenicity of diesel exhaust. Although the Waller (1981) study

had a 25-year follow-up period, the cohort was restricted to only employees (ages 45 to 64) currently in service. Employees who left the job earlier, as well as those who were still employed after age 64 and who may have died from cancer, were excluded.

Wong et al. (1985) conducted a mortality study of heavy equipment operators that demonstrated a significant increased risk of liver cancer in total and in various subcohorts. The same analysis also showed statistically significant deficits in cancers of the large intestine and rectum. Metastasis from the cancers of the large intestine and rectum in the liver probably were misclassified as primary liver cancer which led to an observed excess risk. This study did demonstrate a nonsignificant positive trend for cancer of the lung with length of membership and latency. Analysis of deceased retirees showed a significant excess of lung cancer. Individuals without work histories who started work prior to 1967 when records were not kept may have been in the same jobs for the longest period of time. Workers without job histories included those who had the same job before and after 1967 and thus may have worked about 12 to 14 years longer; these workers exhibited significant excess risks of lung cancer and stomach cancer. If this assumption about duration of jobs is correct, then these site-specific causes can be linked to diesel exhaust exposure. One of the methodological limitations of this study is that most of these men worked outdoors; thus, this cohort might have had relatively low exposure to diesel exhaust. The authors did not present any environmental measurement data either. Because of the absence of detailed work histories for 30% of the cohort and the availability of only partial work histories for the remaining 70%, jobs were classified and ranked according to presumed diesel exposure. Information is lacking regarding duration of employment in the job categories (used for surrogate of exposure) and other confounding factors (alcohol consumption, cigarette smoking, etc.). Thus, this study cannot be used to support a causal association or to refute the same between exposure to diesel exhaust and lung cancer.

A 2-year mortality analysis by Boffetta and Stellman (1988), of the American Cancer Society's prospective study, after controlling for age and smoking, demonstrated an excess risk of lung cancer in certain occupations with potential exposure to diesel exhaust. These excesses were statistically significant among miners (RR = 2.67, 95% CI = 1.63, 4.37) and heavy equipment operators (RR = 2.6, 95% CI = 1.12, 6.06). The elevated risks were nonsignificant in railroad workers (RR = 1.59) and truck drivers (RR = 1.24). A dose

response was also observed for truck drivers. With the exception of miners, exposure to diesel exhaust occurred in the three other occupations showing an increase in the risk of lung cancer. Despite methodologic limitations such as the lack of representiveness of the study population (composed of volunteers only who were probably healthier than the general population) leading to an underestimation of the risk and the questionable reliability of exposure data based on self-administered questionnaires that were not validated, this study is suggestive of a causal association between exposure to diesel exhaust and excess risk of lung cancer.

Two mortality studies of railroad workers were conducted by Howe et al. (1983) in Canada and Garshick et al. (1988) in the United States. The Canadian study found relative risks of 1.2 ($p < 0.01$) and 1.35 ($p < 0.001$) among "possibly" and "probably" exposed groups, respectively. The trend test showed a highly significant dose-response relationship with exposure to diesel exhaust and the risk of lung cancer. The main limitation of the study was the inability to separate overlapping exposures of coal dust and diesel fumes. Information on jobs was available at retirement only. There was also insufficient detail on the classification of jobs by diesel exhaust exposure. The exposures could have been nonconcurrent or concurrent, but since the data are lacking, it is possible that the observed excess could be due to the effect of both coal dust and diesel fumes and not due to just one or the other. However, it should be noted that, so far, coal dust has not been demonstrated to be a pulmonary carcinogen in studies of coal miners, but lack of data on confounders such as asbestos and smoking makes interpretation of this study difficult. When three diesel exhaust exposure categories were examined for smoking-related diseases such as emphysema, laryngeal cancer, esophageal cancer, and buccal cancer, positive trends were observed, raising a possibility that the dose-response demonstrated for diesel exposure may have been due to smoking. The findings of this study are at best suggestive of diesel exhaust being a lung carcinogen.

The most definitive evidence for linking diesel exhaust exposure to lung cancer comes from the Garshick et al. (1988) railroad worker study conducted in the United States. Relative risks of 1.57 (95% CI = 1.19, 2.06) and 1.34 (95% CI = 1.02, 1.76) were found for ages 40 to 44 and 45 to 49, respectively, after the exclusion of workers exposed to asbestos. This study also found that the risk of lung cancer increased with increasing

duration of employment. As this was a large cohort study with lengthy follow-up and adequate analysis, including dose response (based on duration of employment as a surrogate) as well as adjustment for other confounding factors such as asbestos, the observed association between increased lung cancer and exposure to diesel exhaust is more meaningful.

### 8.5.2 Case-Control Studies of Lung Cancer

Among the eight lung cancer case-control studies reviewed in this chapter, only one, the study by Lerchen et al. (1987), did not find an increased risk of lung cancer, after adjusting for age and smoking, for diesel fume exposure. The major limitation of this study was a lack of adequate exposure data derived from the job titles obtained from occupational histories. Next of kin provided the occupational histories for 50% of the cases that were not validated. The power of the study was small (analysis done on males only, 333 cases). On the other hand, statistically nonsignificant excess risks were observed for diesel exhaust exposure by Williams et al. (1977) in railroad workers (OR = 1.4) and truck drivers (OR = 1.34), by Hall and Wynder (1984) in workers who were exposed to diesel exhaust versus those who were not (OR = 1.4 and 1.7 with two different criteria), and by Damber and Larsson (1987) in professional drivers (OR = 1.2). These rates were adjusted for age and smoking. Both Williams et al. (1977) and Hall and Wynder (1984) had high nonparticipation rates of 47 and 36%, respectively. Therefore, the positive results found in these studies are underestimated at the best. In addition, the self-reported exposures used in the study by Hall and Wynder (1984) were not validated. This study also had low power to detect excess risk of lung cancer for specific occupations.

The study by Benhamou et al. (1988), after adjusting for smoking, found significantly increased risks of lung cancer among French motor vehicle drivers (RR = 1.42) and transport equipment operators (RR = 1.35). The main limitation of the study was the inability to separate the exposures to diesel exhaust from those of gasoline exhausts since both motor vehicle drivers and transport equipment operators probably were exposed to the exhausts of both types of vehicles.

Hayes et al. (1989) combined data from three studies (conducted in three different states) to increase the power to detect an association between lung cancer and occupations with a high potential for exposure to diesel exhaust. They found that truck drivers employed

for more than 10 years had a significantly increased risk of lung cancer (OR = 1.5, 95% CI = 1.1, 1.9). This study also found a significant trend of increasing risk of lung cancer with increasing duration of employment among truck drivers. The relative odds were computed by adjusting for birth cohort, smoking, and state of residence. The main limitation of this study is again the mixed exposures to diesel and gasoline exhausts, since information on type of engine was lacking. Also, potential bias may have been introduced because the way in which the cause of death was ascertained for the selection of cases varied in the three studies. Further, the methods used in these studies to classify occupational categories were different probably leading to incompatibility of occupational categories.

The most convincing evidence comes from the Garshick et al. (1987) case-control study of railroad workers and the Steenland et al. (1990) case-control study of truck drivers in the Teamsters Union. Garshick et al. found that after adjustment for asbestos and smoking, the relative odds for continuous exposure were 1.39 (95% CI = 1.05, 1.83). Among the younger workers with longer diesel exhaust exposure, the risk of lung cancer increased with the duration of exposure after adjusting for asbestos and smoking. Even after the exclusion of recent diesel exhaust exposure (5 years before death), the relative odds increased to 1.43 (95% CI = 1.06, 1.94). This study appears to be a well-conducted and well-analyzed case-control study with reasonably good power. Potential confounders were controlled adequately, and interactions between diesel exhaust and other lung cancer risk factors were tested.

Steenland et al. (1990), on the other hand, created two separate work history files, one from Teamsters Union pension files and the other from next-of-kin interviews. Using duration of employment as a categorical variable and considering employment after 1959 (when presumed dieselization occurred) for long haul drivers, the risk of lung cancer increased with increasing years of exposure. Using 1964 as the cut-off, a similar trend was observed for long haul drivers. For short haul drivers the trend was positive with a 1959 cut-off but not when 1964 was used as the cut-off. For truck drivers who primarily drove diesel trucks and worked for 35 years, the relative odds were 1.89. The limitations of this study include possible misclassifications of exposure and smoking, lack of levels of diesel exposure, smaller nonexposed group, and insufficient latency period. Given these limitations, the findings of this study are probably underestimated.

### 8.5.3 Case-Control Studies of Bladder Cancer

Of the seven bladder cancer case-control studies, four studies found increased risk in occupations with a high potential diesel exhaust exposure. A significantly increased risk of bladder cancer was found in Canadian railroad workers (RR = 9.0, 95% CI = 1.2, 349.5; Howe et al., 1980), truck drivers from New Hampshire and Vermont (OR = 2.9, $p < 0.05$; Hoar and Hoover, 1985), and in Argentinean truck and railroad drivers (RR = 4.31, $p < 0.002$; Iscovich et al., 1987). A positive trend with increasing employment as truck driver ($p = 0.006$) was observed by Hoar and Hoover, 1985 in their study of truck drivers from New Hampshire and Vermont. Significantly increased risks also were observed with increasing duration of employment of $\geq$20 years in truck drivers (OR = 12, $p = 0.01$) and railroad workers (OR = 2.21, $p < 0.05$; Steenland et al., 1987). No significant increased risk was found for any diesel-related occupations in studies by Wynder et al. (1985), Iyer et al. (1990), or Steineck et al. (1990). All these studies had several limitations including inadequate characterization of diesel exhaust exposure, lack of validation of surrogate measures of exposure, and presence of other confounding factors (cigarette smoking, urinary retention, concentrated smoke within the truck cab, etc.); most of them had small sample sizes, and none presented any latency analysis.

### 8.5.4 Relevant Methodologic Issues

Throughout this chapter various methodologic limitations of individual studies have been discussed, such as small sample size, short follow-up period, lack of latency analysis, and lack of data on confounding variables. However, two of the major methodologic concerns in these studies are use of death certificates to determine the cause of death and lack of data on cigarette smoking which is a strong risk factor for both lung cancer and bladder cancer. Death certificates were used by all of the seven cohort mortality studies, two case-control studies of lung cancer, and one case-control study of bladder cancer, to determine cause of death. Use of death certificates could lead to misclassification bias. Studies of autopsies done between 1960 and 1971 demonstrated that lung cancer was overdiagnosed when compared to hospital discharge with no incidental cases found at autopsy (Rosenblatt et al., 1971). Schottenfeld et al. (1982) also found an overdiagnosis of lung cancer among autopsies conducted in 1977 and 1978. On the other hand, Percy et al. (1981)

noted 95% concordance when comparing 10,000 lung cancer deaths observed in the Third National Cancer Survey during 1969 to 1971 (over 90% were confirmed histologically) to death certificate-coded cause of death. For bladder cancer, the concordance rate was 91%. These more recent findings suggest that the diagnosis of lung cancer as well as bladder cancer on death certificates is better than anticipated. Furthermore, an overdiagnosis of lung cancer or bladder cancer on death certificates would reduce the ability of the study to detect an effect of diesel exhaust exposure.

All the cohort studies considered for this report are retrospective mortality studies. It is usually difficult to obtain smoking history in such instances. The smoking histories obtained from surrogates (next of kin being either a spouse or an offspring) were found to be accurate by Lerchen and Samet (1986) and McLaughlin et al. (1987). Lerchen and Samet (1986) did not detect any consistent bias in the report of cigarette consumption. In contrast, overreporting of cigarette smoking by surrogates was observed by Rogot and Reid (1975), Kolonel et al. (1977), and Humble et al. (1984). Kolonel et al. (1977) found that the age at which an individual started smoking was reported within 4 years of actual age 84% of the time. The indication from these studies is that surrogates were able to provide fairly credible information on the smoking habits of the study subjects. If the surrogates of the cases were more likely to overreport cigarette smoking as compared to the controls, then it might be harder to find an effect of diesel exhaust because most of the increase in lung cancer would be attributed to smoking rather than to the effect of exposure to diesel exhaust.

### 8.5.5 Criteria of Causal Inference

In most situations epidemiologic data are used to delineate the causality of certain health effects. Several cancers have been causally associated with exposure to agents for which there is no direct biological evidence. Insufficient knowledge about the biological basis for diseases in humans makes it difficult to identify exposure to an agent as causal, particularly for malignant diseases when the exposure was in the distant past. Consequently, epidemiologists and biologists have provided a set of criteria that define a causal relationship between exposure and the health outcome. A causal interpretation is enhanced for studies that meet these criteria. None of these criteria actually proves causality; actual proof is rarely attainable when dealing with environmental carcinogens. None of these criteria should

be considered either necessary (except temporality of exposure) or sufficient in itself. The absence of any one or even several of these criteria does not prevent a causal interpretation. However, if more criteria apply it provides credible evidence for causality.

Thus, applying the criteria of causal inference to the seven cohort mortality and eight case-control studies in which risk of lung cancer was assessed, resulted in the following:

- Temporality: There is a temporality of exposure to diesel exhaust prior to the occurrence of lung cancer.

- Strength of Association: The strength of association between exposure and the occurrence of lung cancer in the cohort studies showed a 30 to 57% higher risk among exposed as compared to nonexposed (Howe et al., 1983; Wong et al., 1985; Boffetta and Stellman, 1988; Garshick et al., 1988). In case-control studies, the risk varied from 20 to 89% higher among exposed as compared to nonexposed (Williams et al., 1977; Hall and Wynder, 1984; Damber and Larsson, 1987; Garshick et al., 1987; Benhamou et al., 1988; Hayes et al., 1989; Steenland et al., 1990). Some of these studies did adjust for the confounding effects of smoking, asbestos, and other exposures.

- Consistency: Four cohort studies and seven case-control studies of lung cancer conducted in several populations in the United States and Europe consistently found the same effect (i.e., lung cancer).

- Specificity: All of the above-mentioned studies found the same specific effect (i.e., lung cancer).

- Biological Gradient: The biological gradient, which refers to the dose-response relationship, was observed in the cohorts of Canadian railway workers (Howe et al., 1983), heavy bulldozer operators (Wong et al., 1985), and truck drivers who had enrolled in the American Cancer Society's prospective mortality study (Boffetta and Stellman, 1988). In the case-control studies, a dose response was observed in railroad workers (Garshick et al., 1988; Hayes et al., 1989; Steenland et al., 1990). Although other studies failed to observe a dose response, these studies were methodologically limited due to confounding by other exposures and lack of either quantitative data on exposure or surrogate data on dose.

- Biological Plausibility: Because diesel exhaust particles consist of a carbon core with surface layers of organics, the tumorigenic activity either resides in one or both of these components. As explained in Chapter 9, there is clear evidence that the organic constituents have the capacity to interact with DNA and give rise to mutations, chromosomal aberrations, and cell transformations, all well-established steps in the process of carcinogenesis. Furthermore, these organic chemicals include a variety of polycyclic aromatic hydrocarbons (PAHs) and nitroaromatics, many of which are known to be pulmonary carcinogens. Alternatively, Vostal (1986) suggests that "diesel" particles themselves induce lung cancer, most likely

via an epigenetic mechanism, if they are present at sufficiently high doses. This makes a convincing argument for biological plausibility of lung cancer occurrence.

When the same causal inference criteria were applied to the seven case-control studies in which risk of bladder cancer was assessed, the results were:

- Temporality: There is temporality of exposure to diesel exhaust prior to the occurrence of bladder cancer.

- Strength of Association: The relative odds of getting bladder cancer among exposed as compared to nonexposed ranged from 2 to 12 times higher (Howe et al., 1980; Hoar and Hoover, 1985; Iscovich et al., 1987; Steenland et al., 1987). None of these studies adjusted for other confounding effects such as cigarette smoking, exposures to other chemicals, urinary retention, etc.

- Consistency: Four out of seven bladder case-control studies conducted in the United States and abroad found an increased relative odds of bladder cancer in the exposed population. None of the cohort studies showed increased bladder cancer mortality; however, people rarely die from bladder cancer, so bladder cancer excess is unlikely to be detected in mortality studies.

- Specificity: Four out of seven case-control studies found an excess of bladder cancer. The specificity criterion, per se, does not apply in this particular instance since these are case-control studies.

- Biological Gradient: Dose response was observed in two out of four studies showing increasing relative odds with increasing length of employment (Hoar and Hoover, 1985; Steenland et al., 1987).

- Biological Plausibility: It has been demonstrated that motor exhaust emissions contain PAHs and nitro-PAHs (Stenberg et al., 1983; Rosenkranz and Mermelstein, 1983). There is some evidence that nitro-PAHs may be responsible for the induction of human bladder cancer. Nitro-PAHs can be metabolized to aromatic amine derivatives, and some of these agents are known to be capable of inducing urinary bladder cancer (Clayson and Garner, 1976). Furthermore, 1-nitropyrene (1-NP) has been reported to be carcinogenic in the rat mammary gland (Hirose et al., 1984); the structurally related 4-aminobiphenyl, which induces bladder cancer in humans, also induces mammary gland tumors in rats (Hirose et al., 1984). Although the applicability of these experimental results to humans is unknown, the laboratory evidence certainly suggests the biological plausibility of diesel exhaust to be a urinary bladder carcinogen.

In summary, although some of the causality inference criteria do apply to bladder cancer, the evidence for bladder cancer in populations exposed to diesel exhaust is inadequate. On the other hand, all the causality inference criteria apply well for lung cancer.

An excess risk of lung cancer was observed in four out of seven cohort studies and seven out of eight case-control studies. Dose response was found in three cohort studies and three case-control studies using duration of employment as a surrogate for dose. However, because of lack of the actual data on exposure to diesel exhaust in these studies and other methodologic limitations, such as insufficient latency for lung cancer to develop, the human evidence falls short of being sufficient, and hence is considered to be limited for diesel exhaust exposure.

# REFERENCES


Ahlberg, J.; Ahlbom, A.; Lipping, H.; Norell, S.; Österblom, L. (1981) [Cancer among professional drivers—a problem-oriented register-based study]. Läkartidningen 78: 1545-1546.

Benhamou, S.; Benhamou, E.; Flamant, R. (1988) Occupational risk factors of lung cancer in a French case-control study. Br. J. Ind. Med. 45: 231-233.

Boffetta, P.; Stellman, S. D. (1988) Association between diesel exhaust exposure and multiple myeloma: an example of confounding. Prev. Med. 17: 236-237.

Buiatti, E.; Kriebel, D.; Geddes, M.; Santucci, M.; Pucci, N. (1985) A case control study of lung cancer in Florence, Italy. I. Occupational risk factors. J. Epidemiol. Commun. Health 39: 244-250.

Clayson, D. B.; Garner, R. C. (1976) Carcinogenic aromatic amines and related compounds. In: Searle, C. E., ed. Chemical carcinogens. Washington, DC: American Chemical Society. (ACS monograph no. 173).

Coggon, D.; Pannett, B.; Acheson, E. D. (1984) Use of job-exposure matrix in an occupational analysis of lung and bladder cancers on the basis of death certificates. JNCI J. Natl. Cancer Inst. 72: 61-65.

Damber, L. A.; Larsson, L. G. (1987) Occupation and male lung cancer: a case-control study in northern Sweden. Br. J. Ind. Med. 44: 446-453.

Daniel, J. H. (1984) The use of diesel-powered equipment in U.S. underground coal operations. Presented at: American Mining Congress international coal show; May; Chicago, IL. Washington, DC: Department of the Interior, Bureau of Mines.

Edling, C.; Anjou, C.-G.; Axelson, O.; Kling, H. (1987) Mortality among personnel exposed to diesel exhaust. Int. Arch. Occup. Environ. Health 59: 559-565.

Flodin, U.; Fredriksson, M.; Persson, B. (1987) Multiple myeloma and engine exhausts, fresh wood, and creosote: a case-referent study. Am. J. Ind. Med. 12: 519-529.

Garland, F. C.; Gorham, E. D.; Garland, C. F.; Ducatman, A. M. (1988) Testicular cancer in US Navy personnel. Am. J. Epidemiol. 127: 411-414.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1987) A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 135: 1242-1248.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1988) A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 137: 820-825.

Gustafsson, L.; Wall, S.; Larsson, L.-G.; Skog, B. (1986) Mortality and cancer incidence among Swedish dock workers—a retrospective cohort study. Scand. J. Work Environ. Health 12: 22-26.

Gustavsson, P.; Plato, N.; Lidström, E.-B.; Hogstedt, C. (1990) Lung cancer and exposure to diesel exhaust among bus garage workers. Scand. J. Work Environ. Health 16: 348-354.

Hall, N. E. L.; Wynder, E. L. (1984) Diesel exhaust exposure and lung cancer: a case-control study. Environ. Res. 34: 77-86.

Harris, J. E. (1983) Diesel emissions and lung cancer. Risk Anal. 3: 83-100.

Hayes, R. B.; Thomas, T.; Silverman, D. T.; Vineis, P.; Blot, W. J.; Mason, T. J.; Pickle, L. W.; Correa, P.; Fontham, E. T. H.; Schoenberg, J. B. (1989) Lung cancer in motor exhaust-related occupations. Am. J. Ind. Med. 16: 685-695.

Hirose, M.; Lee, M.-S.; Wang, C. Y.; King, C. M. (1984) Induction of rat mammary gland tumors by 1-nitropyrene, a recently recognized environmental mutagen. Cancer Res. 44: 1158-1162.

Hoar, S. K.; Hoover, R. (1985) Truck driving and bladder cancer mortality in rural New England. JNCI J. Natl. Cancer Inst. 74: 771-774.

Howe, G. R.; Burch, J. D.; Miller, A. B.; Cook, G. M.; Esteve, J.; Morrison, B.; Gordon, P.; Chambers, L. W.; Fodor, G.; Winsor, G. M. (1980) Tobacco use, occupation, coffee, various nutrients, and bladder cancer. JNCI J. Natl. Cancer Inst. 64: 701-713.

Howe, G. R.; Fraser, D.; Lindsay, J.; Presnal, B.; Yu, S. Z. (1983) Cancer mortality (1965-77) in relation to diesel fume and coal exposure in a cohort of retired railway workers. JNCI J. Natl. Cancer Inst. 70: 1015-1019.

Humble, C. G.; Samet, J. M.; Skipper, B. E. (1984) Comparison of self- and surrogate-reported dietary information. Am. J. Epidemiol. 119: 86-98.

Iscovich, J.; Castelleto, R.; Estevè, J.; Muñoz, N.; Colanzi, R.; Coronel, A.; Deamezola, I.; Tassi, V.; Arslan, A. (1987) Tobacco smoking, occupational exposure and bladder cancer in Argentina. Int. J. Cancer 40: 734-740.

Iyer, V.; Harris, R. E.; Wynder, E. L. (1990) Diesel exhaust exposure and bladder cancer risk. Eur. J. Epidemiol. 6: 49-54.

Jensen, O. M.; Wahrendorf, J.; Knudsen, J. B.; Sørensen, B. L. (1987) The Copenhagen case-referent study on bladder cancer: risks among drivers, painters, and certain other occupations. Scand. J. Work Environ. Health 13: 129-134.

Kaplan, I. (1959) Relationship of noxious gases to carcinoma of the lung in railroad workers. JAMA J. Am. Med. Assoc. 171: 2039-2043.

Kolonel, L. N.; Hirohata, T.; Nomura, A. M. Y. (1977) Adequacy of survey data collected from substitute respondents. Am. J. Epidemiol. 106: 476-484.

Lerchen, M. L.; Samet, J. M. (1986) An assessment of the validity of questionnaire responses provided by a surviving spouse. Am. J. Epidemiol. 123: 481-489.

Lerchen, M. L.; Wiggins, C. L.; Samet, J. M. (1987) Lung cancer and occupation in New Mexico. JNCI J. Natl. Cancer Inst. 79: 639-645.

McLaughlin, J. K.; Dietz, M. S.; Mehl, E. S.; Blot, W. J. (1987) Reliability of surrogate information on cigarette smoking by type of informant. Am. J. Epidemiol. 126: 144-146.

Percy, C.; Stanek, E., III; Gloeckler, L. (1981) Accuracy of cancer death certificates and its effect on cancer mortality statistics. Am. J. Public Health 71: 242-250.

Raffle, P. A. B. (1957) The health of the worker. Br. J. Ind. Med. 14: 73-80.

Risch, H. A.; Burch, J. D.; Miller, A. B.; Hill, G. B.; Steele, R.; Howe, G. R. (1988) Occupational factors and the incidence of cancer of the bladder in Canada. Br. J. Ind. Med. 45: 361-367.

Rogot, E.; Reid, D. D. (1975) The validity of data from next-of-kin in studies of mortality among migrants. Int. J. Epidemiol. 4: 51-54.

Rosenblatt, M. B.; Teng, P. K.; Kerpe, S.; Beck, I. (1971) Causes of death in 1,000 consecutive autopsies. N. Y. State J. Med. 71: 2189-2193.

Rosenkranz, H. S.; Mermelstein, R. (1983) Mutagenicity and genotoxicity of nitroarenes: all nitro-containing chemicals were not created equal. Mutat. Res. 114: 217-267.

Rushton, L.; Alderson, M. R.; Nagarajah, C. R. (1983) Epidemiological survey of maintenance workers in London Transport Executive bus garages at Chiswick Works. Br. J. Ind. Med. 40: 340-345.

Schenker, M. B.; Smith, T.; Munoz, A.; Woskie, S.; Speizer, F. E. (1984) Diesel exposure and mortality among railway workers: results of a pilot study. Br. J. Ind. Med. 41: 320-327.

Schottenfeld, D.; Eaton, M.; Sommers, S. C.; Alonso, D. R.; Wilkinson, C. (1982) The autopsy as a measure of accuracy of the death certificate. Bull. N. Y. Acad. Med. 58: 778-794.

Siemiatycki, J.; Gérin, M.; Stewart, P.; Nadon, L.; Dewar, R.; Richardson, L. (1988) Associations between several sites of cancer and ten types of exhaust and combustion products: results from a case-referent study in Montreal. Scand. J. Work Environ. Health 14: 79-90.

Silverman, D. T.; Hoover, R. N.; Albert, S.; Graff, K. M. (1983) Occupation and cancer of the lower urinary tract in Detroit. JNCI J. Natl. Cancer Inst. 70: 237-245.

Silverman, D. T.; Hoover, R. N.; Mason, T. J.; Swanson, G. M. (1986) Motor exhaust-related occupations and bladder cancer. Cancer Res. 46: 2113-2116.

Steenland, K. (1986) Lung cancer and diesel exhaust: a review. Am. J. Ind. Med. 10: 177-189.

Steenland, K.; Burnett, C.; Osoria, A. M. (1987) A case-control study of bladder cancer using city directories as a source of occupational data. Am. J. Epidemiol. 126: 247-257.

Steenland, N. K.; Silverman, D. T.; Hornung, R. W. (1990) Case-control study of lung cancer and truck driving in the Teamsters Union. Am. J. Public Health 80: 670-674.

Steineck, G.; Plato, N.; Gerhardsson, M.; Norell, S. E.; Hogstedt, C. (1990) Increased risk of urothelial cancer in Stockholm during 1985-87 after exposure to benzene and exhausts. Int. J. Cancer 45: 1012-1017.

Stenberg, U.; Alsberg, T.; Westerholm, R. (1983) Emission of carcinogenic components with automobile exhausts. Environ. Health Perspect. 47: 53-63.

Stern, F. B.; Lemen, R. A.; Curtis, R. A. (1981) Exposure of motor vehicle examiners to carbon monoxide: a historical prospective mortality study. Arch. Environ. Health 36: 59-66.

Vineis, P.; Magnani, C. (1985) Occupation and bladder cancer in males: a case-control study. Int. J. Cancer 35: 599-606.

Vostal, J. J. (1986) Factors limiting the evidence for chemical carcinogenicity of diesel emissions in long-term inhalation experiments. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 381-396. (Developments in toxicology and environmental science: v. 13).

Waller, R. E. (1981) Trends in lung cancer in London in relation to exposure to diesel fumes. Environ. Int. 5: 479-483.

Waxweiler, R. J.; Wagoner, J. K.; Archer, V. E. (1973) Mortality of potash workers. J. Occup. Med. 15: 486-489.

Williams, R. R.; Stegens, N. L.; Goldsmith, J. R. (1977) Associations of cancer site and type with occupation and industry from the Third National Cancer Survey interview. J. Natl. Cancer Inst. 59: 1147-1185.

Wong, O.; Morgan, R. W.; Kheifets, L.; Larson, S. R.; Whorton, M. D. (1985) Mortality among members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. Br. J. Ind. Med. 42: 435-448.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garshick, E.; Speizer, F. E. (1988a) Estimation of the diesel exhaust exposures of railroad workers: I. current exposures. Am. J. Ind. Med. 13: 381-394.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garschick, E.; Speizer, F. E. (1988b) Estimation of the diesel exhaust exposures of railroad workers: II. national and historical exposures. Am. J. Ind. Med. 13: 395-404.

Wynder, L. E.; Dieck, G.; Hall, N. E. (1985) A case control study of diesel exhaust exposure and bladder cancer. Environ. Res. 34: 475-489.

# ADDENDUM TO CHAPTER 8

Since the last update of this document, eight epidemiologic studies have been published. Of these studies, four were excluded from this review. Three studies (Swanson et al., 1993; Cordier et al., 1993; and Notani et al., 1993) were hypothesis-generating studies and would not have contributed anything towards the evaluation of the carcinogenicity of diesel exhaust. The fourth study (Guberan et al., 1992), which failed to distinguish between diesel exhaust and gasoline exhaust, was excluded due to uncertain contribution of diesel exhaust to the observed increase for lung cancer.

The review of the remaining four studies appears in this addendum.

## Gustavsson et al. (1990): Lung Cancer and Exposure to Diesel Exhaust Among Bus Garage Workers

A retrospective mortality study (from 1952 to 1986), cancer incidence study (from 1958 to 1984), and nested case-control study were conducted among a cohort of 708 male workers from five bus garages in Stockholm, Sweden, who had worked for at least 6 mo between 1945 and 1970. Thirteen individuals were lost to follow-up, reducing the cohort to 695.

Information was available on location of workplace, job type, and beginning and ending of work periods. Workers were traced using a computerized register of the living population, death and burial books, and data from the Stockholm City archives.

For the cohort mortality analyses, death rates of the general population of greater Stockholm were used. Death rates of occupationally active individuals, a subset of the general population of greater Stockholm, were used as a second comparison group to reduce the bias from "healthy worker effect". Mortality analysis was conducted using the "occupational mortality analysis program" (OCMAP-PC). For cancer incidence analysis, the "epidemiology in Linköping" (EPILIN) program was utilized using the incidence rates obtained from the cancer registry.

For the nested case-control study, both dead and incident primary lung cancers, identified in the register of cause of deaths and the cancer register, were selected as cases (20). Six controls matched on age $\pm 2$ years, selected from the noncases at the time of the diagnosis of cases, were drawn at random without replacements. Matched analyses were

done to calculate odds ratios using conditional logistic regression. The EGRET and Epilog programs were used for these analyses.

Diesel exhaust and asbestos exposure assessments were performed by industrial hygienists based on the intensity of exposure to diesel exhaust and asbestos, specific for workplace, work task, and calendar time period. A diesel exhaust exposure assessment was based on (1) amount of emission (number of buses, engine size, running time, and type of fuel), (2) ventilatory equipment and air volume of the garages, and (3) job types and work practices. Based on detailed historical data and very few actual measurements, relative exposures were estimated (these were not absolute exposure levels). The scale was set to 0 for unexposed and 1 for lowest exposure with each additional unit increase corresponding to a 50% increase in successive intensity (i.e., 1.5, 2.25, 3.38, and 5.06).

Based on personal sampling of asbestos during 1987, exposures were estimated and time-weighted annual mean exposures were classified on a scale of three degrees (0, 1, and 2). Cumulative exposures for both diesel exhaust and asbestos were calculated by multiplying the level of exposure by the duration of every work period. An exposure index was calculated by adding for every individual contributions from all work periods for both diesel exhaust and asbestos. Four diesel exhaust index classes were created: (1) 0 to 10, (2) 10 to 20, (3) 20 to 30, and (4) >30. The four asbestos index classes were (1) 0 to 20, (2) 20 to 40, (3) 40 to 60, and (4) >60. The cumulative exposure indices were used for the nested case-control study.

Excesses were observed for all cancers and some other site-specific cancers using both comparison populations for the cohort mortality study—but none of them was statistically significant. Based on 17 cases, standardized mortality ratios (SMRs) for lung cancer were 122 and 115 using Stockholm occupationally active and general population, respectively. No dose-response was observed with increasing cumulative exposure. The cancer incidence study reportedly confirmed the mortality results (results not given).

The nested case-control study showed increasing risk of lung cancer with increasing exposure. Weighted linear regression gave RRs of 1.34 (95% CI = 1.09 to 1.64), 1.81 (95% CI = 1.20 to 2.71), and 2.43 (95% CI = 1.32 to 4.47) for the diesel exhaust indices 10 to 20, 20 to 30, and >30, respectively, using 0 to 10 as the comparison group. The results from conditional logistic regression were similar to those obtained by weighted

linear regression but none was statistically significant. Adjustment for asbestos exposure did not change the lung cancer risk for diesel exhaust.

The main strength of this study is the detailed exposure matrices constructed for both diesel exhaust and asbestos exposure, although they were based primarily on job tasks and very few actual measurements. There are a few methodological limitations to this study. The cohort is small and there were only 17 lung cancer deaths, thus the power is low. Exposure or outcome may be misclassified, although any resulting bias in the relative risk estimates is likely to be towards unity, because exposure classification was done independently of the outcome. Although the analysis by dose indices was done, no latency analysis was performed. Finally, data on smoking were missing, thus potentially confounding the lung cancer results. The authors suggest that even the heaviest smoking among individuals who were heavily exposed to diesel exhaust will be unable to explain the excess relative risk of 2.4 observed in this group. This may be an overstatement, however, as cigarette smoking is a very strong risk factor for lung cancer. Overall, this study provides some support to the excess lung cancer results found earlier among populations exposed to diesel exhaust.

## Boffetta et al. (1990): Case-Control Study on Occupational Exposure to Diesel Exhaust and Lung Cancer Risk

This is an ongoing (since 1969) case-control study of tobacco-related diseases in 18 hospitals (six U.S. cities). Cases comprise 2,584 males, with histologically confirmed primary lung cancers. Sixty-nine cases were matched to one control, whereas 2,515 were matched to two controls. Controls were individuals who were diagnosed with non-tobacco-related diseases. The matching was done for sex, age ($\pm 2$ years), hospital, and year of interview. The interviews were conducted at the hospitals at the time of diagnosis. In 1985, the occupational section of the questionnaire was modified to include the usual occupation and up to five other jobs as well as duration (in years) worked in those jobs. After 1985, information was also obtained on exposure to 45 groups of chemicals including diesel exhaust at the workplace or during hobby activities. A priori aggregation of occupations was categorized into low probability of diesel exhaust exposure (reference group), possible exposure (19 occupations), and probable exposure (13 occupations). Analysis was conducted

based on "usual occupation" on all study subjects and any occupation with sufficient cases was eligible for further analysis. In addition, cases enrolled after 1985 for which there were self-reported diesel exhaust exposure and detailed work histories were also analyzed separately.

Both matched and unmatched analyses were done by calculating the adjusted (for smoking and education) relative odds using the Mantel-Haenzael method and calculating the test-based 95% confidence interval using the Miettinen method. Unconditional logistic regression was used to adjust for potential confounders (the PROC LOGIST of SAS). Linear trends for risk were also tested according to Mantel.

Adjusted relative odds for possible and probable exposure groups as well as the truck drivers were slightly below unity, none being statistically significant for the entire study population. Although slight excesses were observed for the self-reported diesel exhaust exposure group and the subset of post-1985 enrollees for highest duration of exposure (for self-reported exposure, occupations with probable exposure and for truck drivers), none was statistically significant. Trend tests for the risk of lung cancer among self-reported diesel exhaust exposure, probable exposure, and truck drivers with increasing exposure (duration of exposure used as surrogate for increasing dose) were nonsignificant too. Statistically significant lung cancer excesses were observed for cigarette smoking only.

The major strength of this study is availability of detailed smoking history. Even though detailed information was obtained for the usual and five other occupations (1985), no effort was made to estimate or verify the actual exposure to diesel exhaust; instead, duration of employment was used as a surrogate for dose. The numbers of cases and controls were large; however, the number of individuals exposed to diesel exhaust was relatively few, thus reducing the power of the study. This study did not attempt latency analysis either. Given these limitations, the findings of this study are unable to provide either positive or negative evidence for a causal association between diesel exhaust and occurrence of lung cancer.

## Hansen (1993): A Follow-up Study on the Mortality of Truck Drivers

This is a retrospective cohort mortality study of unskilled male laborers, ages 15 to 74 years, in Denmark, identified from a nationwide census file of November 9, 1970. The exposed group included all truck drivers employed in the road delivery or long-haul business

(14,225). The unexposed group included all laborers in certain selected occupational groups considered to be unexposed to fossil fuel combustion products and to resemble truck drivers in terms of work-related physical demands and various personal background characteristics (43,024).

Through automatic record linkage between the 1970 census register (the Central Population Register 1970 to 1980) and the Death Certificate Register (1970 to 1980), the population was followed for cause-specific mortality or emigration up to November 9, 1980. Expected number of deaths among truck drivers were calculated by using the 5-year age group and 5-year time period death rates of the unexposed group and applying them to the person-years accumulated by truck drivers. International Classification of Diseases Revision 8 was used to code the underlying cause of death. Test based confidence intervals (CI) were calculated using Miettinen's Method. A Poisson distribution was assumed for the smaller numbers and CI were calculated based on exact Poisson distribution (Ciba-Geigy). Total person-years accrued by truck drivers were 138,302, whereas, for the unexposed population, they were 407,780. There were 627 deaths among truck drivers and 3,811 deaths in the unexposed group. Statistically significant (SS) excesses were observed for all cancer mortality (SMR = 121, 95% CI = 104 to 140); cancer of respiratory organs (SMR = 160, 95% CI = 128 to 198), which mainly was due to cancer of bronchus and lung (SMR = 160, 95% CI = 126 to 200); and multiple myeloma (SMR = 439, 95% CI = 142 to 1,024). When lung cancer mortality was further explored by age groups, excesses were observed in most of the age groups (30 to 39, 45 to 99, 50 to 54, 55 to 59, 60 to 64, and 65 to 74), but there were small numbers of deaths in each group when stratified by age, and the excesses were statistically significant for the 55 to 59 (SMR = 229, 95% CI = 138 to 358) and 60 to 64 (SMR = 227, 95% CI = 142 to 344) age groups only.

As acknowledged by the author, the study has quite a few methodologic limitations. The exposure to diesel exhaust is assumed in truck drivers based on diesel-powered trucks, but no validation of qualitative or quantitative exposure is attempted. It is also not known whether any of these truck drivers or any other laborers had changed jobs after the census of November 9, 1970, thus creating potential misclassification bias in exposure to diesel exhaust. The lack of smoking data and a 36% rural population (usually consuming less tobacco) in the unexposed group further confound the lung cancer results. The follow-up

period is relatively short and a latency analysis was not attempted. At best, the findings of this study are consistent with the findings of other truck driver studies.

## Emmelin et al. (1993): Diesel Exhaust Exposure and Smoking: A Case-Referent Study of Lung Cancer Among Swedish Dock Workers

This is a case-control study of lung cancer drawn from the cohort defined as all male workers who had been employed as dock workers for at least 6 mo between 1950 and 1974. In the population of 6,573 from 20 ports, there were 90 lung cancer deaths (cases), identified through Swedish death and cancer registers, during the period of 1960 to 1982. Of these 90 deaths, the 54 who were workers at the 15 ports for which exposure surrogate information was available were chosen for the case-control study. Four controls, matched on port and age, were chosen for each case from the remaining cohort who had survived to the time of diagnosis of the case. Both live and deceased controls were included. The final analyses were done on 50 cases and 154 controls who had complete information on employment dates and smoking data. The smoking strata were created by classifying ex-smokers as nonsmokers if they had not smoked for at least 5 years prior to the date of diagnosis of the case; otherwise they were classified as smokers.

Relative odds and regression coefficients were calculated using conditional logistic regression models. Comparisons were made both with and without smoking included as a variable, and the possible interaction between smoking and diesel exhaust was tested. Both weighted linear regressions of the adjusted relative odds, and the regression coefficients were used to test mortality trends with all three exposure variables.

Exposure to diesel exhaust was assessed indirectly by initially measuring (1) exposure intensity based on exhaust emission, (2) characteristics of the environment in terms of ventilation, and (3) measures of proportion of time in higher exposed jobs. For exhaust emissions annual diesel fuel consumption at a port was used as the surrogate. For ventilation the annual proportion of ships with closed or semi-closed holds was used as the surrogate. The proportion of time spent below decks was used as the surrogate for more exposed jobs. Although data were collected for all three measures only the annual fuel consumption was used for analysis. Because every man was likely to rotate through the various jobs, the authors thought using annual consumption of diesel fuel was the appropriate measure of

exposure. Consequently, in a second analysis, the annual fuel consumption was divided by the number of employees in the same port that year to come up with the fuel-per-person measure, which was further used to create a second measure, "exposed time". The "annual fuel" and exposed-time data were entered in a calendar time-exposure matrix for each port, from which individual exposure measures were created. A third measure, "machine time" (years of employment from first exposure) was also used to compare the results with other studies. All exposure measures were accumulated from the first year of employment or first year of diesel machine use, whichever came later. The last year of exposure was fixed at 1979. All exposures within 2 years prior to the date of lung cancer diagnosis were omitted both from cases and matched controls. A priori classification into three categories of low, medium, and high exposure was done for all three exposure variables, machine time, fuel, and exposed time.

Conditional logistic regression models, adjusting for smoking status, and using low exposures and/or nonsmoker as a comparison group yielded positive trends for all exposure measures, but no trend test results were reported, and only the relative odds for the exposed-time exposure measure in the high-exposure group (OR = 6.8, 90% CI = 1.3 to 34.9), was reported as statistically significant. For smokers, adjusting for diesel exhaust exposure level, the relative odds were statistically significant and about equal for all the three exposure variables—machine time, OR = 5.7 (90% CI = 2.4 to 13.3); fuel, OR = 5.5 (90% CI = 2.4 to 12.7); and exposed time, OR = 6.2 (90% CI = 2.6 to 14.6). Interaction between diesel exhaust and smoking was tested by conditional logistic regression in the exposed-time variable. Although there were positive trends for both smokers and nonsmokers, the trend for smokers was much steeper—low, OR = 3.7 (90% CI = 0.9 to 14.6); medium, OR = 10.7 (90% CI = 1.5 to 78.4); and high, OR = 28.9 (90% CI = 3.5 to 240) indicating more than additive interaction between these two variables.

In the weighted linear regression model with the exposed-time variable the results were similar to those using the logistic regression model. The authors also explored the smoking variable further in various analyses, some of which suggested a strong interaction between diesel exhaust and smoking. However, with just six nonsmokers and no further categorization of smoking amount or duration, these results are of limited value.

The diesel exhaust exposure matrices created using three different variables are

intricate. Analyses by any of these variables essentially yield the same positive results and positive trends, providing consistent support for a real effect of diesel exhaust exposure, at least in smokers. However, there are some methodological limitations to this study which prevent a more definitive conclusion. The numbers of cases and controls are small. There are very few nonsmokers, thus testing the effects of diesel exhaust exposure in them is futile. Lack of information on asbestos exposure, to which dock workers are usually exposed, may also confound the results. Also, no latency analyses are presented. Overall, despite these limitations, this study supports the earlier findings of excess lung cancer mortality among individuals exposed to diesel exhaust.

## Conclusion

In conclusion, of these four recent studies, three provide support to the earlier findings of increased lung cancer among individuals exposed to diesel exhaust, whereas one study is neither able to support nor refute the earlier findings.

## References


Boffetta, P.; Harris, R. E.; Wynder, E. L. (1990) Case-Control study on occupational exposure to diesel exhaust and lung cancer risk. Am. J. Ind. Med. 17:577-591.

Cordier, S.; Clavel, J.; Limasset, J. C.; Boccon-Gibod, L.; Le Moual, N.; Mandereau, L.; Hemon, D. (1993) Occupational risks of bladder cancer in France: A multicenter case-control study. Int. J. Epidemiol. 22:403-411.

Emmelin, A.; Nyström, L.; Wall, S. (1993) Diesel exhaust exposure and smoking: A case-referent study of lung cancer among Swedish Dock workers. Epidemiol. 4:237-244.

Guberan, E.; Usel, M.; Raymond, L.; Bolay, J.; Fioretta, G.; Puissant, J. (1992) Increased risk for lung cancer and for cancer of the gastrointestinal tract among Geneva professional drivers. Br. J. Ind. Med. 49:337-344.

Gustavsson, P.; Plato, N.; Lindström, E-B.; Hogstedt, C. (1990) Lung cancer and exposure to diesel exhaust among bus garage workers. Scand. J. Work. Environ. Health. 16:348-354.

Hansen, E. S. (1993) A follow-up study on the mortality of truck drivers. Am. J. Ind. Med. 23:811-821.

Notani, P. N.; Shah, P.; Jayant, K.; Balakrishnan, V. (1993) Occupation and cancers of the lung and bladder: A case-control study in Bombay. Int. J. Epidemiol. 22:185-191.

Swanson, G. M.; Lin, C-S.; Burns, P. B. (1993) Diversity in the association between occupation and lung cancer among black and white men. Cancer Epidemiol. Biomark Prev. 2:313-320.

# 9. MUTAGENICITY

Since 1978, over 100 publications have appeared in which genotoxicity assays have been employed with diesel emissions, the volatile and particulate fractions (including extracts), or individual chemicals found in diesel emissions. Although most of the studies deal with the question of whether particulate extracts from diesel emissions possessed mutagenic activity in microbial and mammalian cell assays, a number of studies in recent years have employed bioassays (most commonly *Salmonella* TA98 without S9) to evaluate (1) extraction procedures, (2) fuel modifications, (3) bioavailability of chemicals from particles, and (4) exhaust filters or other modifications and other variables associated with diesel emissions. This chapter will focus on the application of the available data to issues of genetic risk assessment; reports dealing with mutagenic activity associated with the metabolism of particular chemicals of diesel particles are discussed in Chapter 10. Also, because of the large number of reports, this discussion will focus on key references. The recent International Agency for Research on Cancer (IARC) monograph (International Agency for Research on Cancer, 1989) contains an exhaustive description of the available studies and other review articles (Claxton, 1983; Pepelko and Peraino, 1983) and the proceedings of several symposia on the health effects of diesel emissions (U.S. Environmental Protection Agency, 1980; Lewtas, 1982; Ishinishi et al., 1986; International Agency for Research on Cancer, 1989) are also available.

## 9.1 GENE MUTATIONS

Huisingh et al. (1978) demonstrated that dichloromethane extracts from diesel particles were mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and without rat liver S9 activation. This first report contained data from several different fractions as well as particulate material from different vehicles and different fuels. Similar results with diesel extracts from various engines and fuels have been reported by a number of investigators using the *Salmonella* frameshift sensitive strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981; Brooks et al., 1984). Similarly,

mutagenic activity was observed in *Salmonella* forward mutation assays measuring 8-azaguanine resistance (Claxton and Kohan, 1981) and in *E. coli* mutation assays (Lewtas, 1983).

An approach to the identification of significant mutagens in chemically complex environmental samples such as diesel exhaust or ambient particulate extracts is the combination of short-term bioassays with chemical fractionation (Scheutzle and Lewtas, 1986). The analysis most frequently is carried out by sequential extraction with increasingly polar or binary solvents. Prefractionation is by silica-column chromatography to separate compounds by polarity, or separation into acidic, basic, and neutral fractions. The resulting fractions are too complex to characterize by chemical methods; however, the bioassay analysis can be used to determine fractions for further analysis. In most of the applications of this concept, *Salmonella* strain TA98 without the addition of S9 has been employed as the indicator for mutagenic activity. Generally, a variety of nitrated polynuclear aromatic compounds has been found, which account for a substantial portion of the mutagenicity found (Liberti et al., 1984; Schuetzle and Frazer, 1986; Schuetzle and Perez,1983). However, not all the bacterial mutagenicity has been identified in this way, and the identity of the remainder of the mutagenic compounds remains unknown. The identity of the nitrated aromatics thus far identified in diesel exhaust was the subject of review in the IARC monograph on diesel exhaust (International Agency for Research on Cancer, 1989).
In addition to the simple qualitative identification of mutagenic chemicals, several investigators have used the numerical data to express mutagenic activity as activity per distance driven or mass of fuel consumed. These types of calculations have been the basis for the estimates that the nitroarenes (both mono- and dinitropyrenes) contribute a significant amount of the total mutagenic activity of the whole extract (Nishioka et al., 1982; Salmeen et al., 1982; Nakagawa et al., 1983). However, as noted by Claxton (1983) because most of these studies used only strain TA98 without exogenous activation, there are several classes of mutagenic chemicals which may have gone undetected.

Matsushita et al. (1986) tested particle-free diesel exhaust gas and a number of benzene nitro-derivatives, and polycyclic aromatic hydrocarbons (PAHs) (many of which have been identified as components of diesel exhaust gas). The particle-free exhaust gas was positive in both TA100 and TA98, but only without S9 activation. Of the 94 nitro-benzene derivatives

tested, 61 were mutagenic, and the majority showed greatest activity in TA100 without S9. Twenty-eight of 50 PAHs tested were mutagenic, all required the addition of S9 for detection, and most appeared to show a stronger response in TA100. When 1,6-dinitropyrene was mixed with various PAHs or an extract of heavy-duty (HD) diesel exhaust, the mutagenic activity in TA98 was greatly reduced when S9 was absent but was increased significantly when S9 was present. These latter results suggested that caution should be used in estimating mutagenicity (or other toxic effects) of complex mixtures from the specific activity of individual components.

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

Morimoto et al. (1986) injected diesel particulate extracts (250 to 4,000 mg/kg) into pregnant Syrian hamsters and measured mutations at the APRT locus in embryo cells cultivated 11 days after injection. Neutral fractions from both light-duty (LD) and HD tar samples resulted in increased mutant frequency at 2,000 and 4,000 mg/kg. Belisario et al. (1984) applied the Ames test to urine from Sprague-Dawley rats exposed to single applications of diesel exhaust particles administered by gastric intubation, ip injection or sc gelatin capsules. In all cases, dose-related increases were seen in TA98 (without and with S9) from urine concentrates taken 24 h after particle administration. Urine from Swiss mice

exposed by inhalation to filtered exhaust (particle concentration 6 to 7 mg/m$^3$) for 7 weeks (Pereira et al., 1981a) or Fischer 344 rats exposed to diesel exhaust particles (2 mg/m$^3$) for 3 mo to 2 years was negative in *Salmonella* strains. Because of the large differences in dosages, these findings should not be construed as conflicting.

Schuler and Niemeier (1981) exposed *Drosophila* males in a stainless steel chamber connected to the 3-m$^3$ chamber used for the chronic animal studies at EPA (see Hinners et al., 1980 for details). Flies were exposed for 8 h and mated to untreated females 2 days later. Althoug the frequency of sex-linked recessive lethals from treated males was not different from controls, the limited sample size precluded detecting less than a threefold increase over controls. The authors also suggested that flies might tolerate exposure to higher concentrations for longer time periods.

Specific-locus mutations were not induced in (C3H $\times$ 101)$F_1$ male mice exposed to diesel exhaust 8 h/day, 7days/week for either 5 or 10 weeks (Russell et al., 1980). The exhaust was a 1:18 dilution and the average particle concentration was 6 mg/m$^3$. After exposure, males were mated to T-Stock females and matings continued for the reproductive life of the males. The results were unequivocally negative; no mutants were detected in 10,635 progeny derived from postspermatogonial cells or in 27,917 progeny derived from spermatogonial cells.

## 9.2 CHROMOSOME EFFECTS

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

extracts from a Nissan diesel engine (Lewtas, 1983) but not by similar extracts from an Oldsmobile diesel engine (Brooks et al., 1984).

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

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

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

In the only studies with mammalian germ cells, Russell et al. (1980) reported no increase in either dominant lethals or heritable translocations in males of T-stock mice exposed by inhalation to diesel emissions. In the dominant lethal test, T-Stock males were exposed for 7.5 weeks and immediately mated to females of different genetic backgrounds (T-stock; [C3H × 101]; [C3H × C57BL/6]; [SEC × C57BL/6]). There were no differences from controls in any of the parameters measured in this assay. For heritable translocation analysis, T-stock males were exposed for 4.5 weeks, mated to (SEC × C57BL/6) females and the $F_1$ males were tested for the presence of heritable translocations. Although no translocations were detected among 358 progeny tested, the historical control incidence is less than 1/1,000.

## 9.3 OTHER GENOTOXIC EFFECTS

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

## 9.4 SUMMARY

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

Structural chromosome aberrations and SCE in mammalian cells have been induced by particles and extracts. Whole exhaust induced micronuclei but not SCE or structural aberrations in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 mo. In a shorter exposure (7 weeks), neither micronuclei nor structural aberrations were

increased in bone marrow of female Swiss mice. Likewise, whole diesel exhaust did not induce dominant lethals or heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

Mutagenicity data have been applied both to issues of heritable genetic risk and somatic cell effects—most notably cancer. For heritable genetic effects, the U.S. Environmental Protection Agency's Guidelines for Mutagenicity Risk Assessment (Federal Register, 1986) are applicable here. The mammalian germ cell studies measuring defined genetic endpoints conducted on diesel emissions have shown negative results; however, the sample size in the heritable translocation test is too small for a meaningful conclusion. In the absence of definitive mammalian germ-cell results, the guidelines recommend that mutagenic activity and the ability to interact with mammalian germ cells be evaluated separately. As stated, the presence of a large number of mutagenic chemicals in diesel emissions is unambiguous. Sperm abnormality assays are presumably the only other source of data on the interaction of diesel emissions with mammalian germ cells. The negative response in the mouse is in apparent conflict with the positive observation in the hamster and there is not sufficient information to resolve this discrepancy. Hence, the questions of germ-cell interaction and the potential for human germ-cell mutagenic risk of diesel emissions remain unanswered.

The application of genotoxicity information to the question of the potential carcinogenicity of chemical agents was initially based on the premise that somatic mutation is an integral step in the carcinogenic process. However, unlike the situation for germ cell mutagenicity, assays are not weighted strictly by their biological relationship to the particular species, sex, or tissue site of cancer. The size of the data base and the degree of correlation of genotoxicity test results with animal cancer bioassays are frequently given great weight. Indeed, a common conclusion of the efforts of the National Toxicology Program on the use of in vitro assays is that no single in vitro genotoxicity test or battery of tests (among the four assays in their program) improve on the performance of the *Salmonella* assay in predicting rodent carcinogenicity of an untested chemical. When rodent carcinogenicity data are available, phylogenetic and other biological aspects of the genotoxicity data are important considerations in the weight-of-evidence process. With diesel emissions, additional complications arise because of the chemical complexity of the material being tested. Although it is clear that several of the individual chemical constituents of diesel exhaust have

been demonstrated to be both mutagenic and carcinogenic, it is likely that the constituents responsible for the mutational increases observed in bacteria are different from those responsible for the observed increases in CHO cells (Li and Dutcher, 1983) or in human hepatoma-derived cells (Eddy et al., 1986). Chapter 10 deals more thoroughly with metabolism and mechanisms of carcinogenesis.

# REFERENCES


Barfknecht, T. R.; Hites, R. A.; Cavaliers, E. L.; Thilly, W. G. (1982) Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency 1981 diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 277-294. (Developments on toxicology and environmental science: v. 10).

Belisario, M. A.; Buonocore, V.; De Marinis, E.; De Lorenzo, F. (1984) Biological availability of mutagenic compounds adsorbed onto diesel exhaust particulate. Mutat. Res. 135: 1-9.

Brooks, A. L.; Li, A. P.; Dutcher, J. S.; Clark, C. R.; Rothenberg, S. J.; Kiyoura, R.; Bechtold, W. E.; McClellan, R. O. (1984) A comparison of genotoxicity of automobile exhaust particles from laboratory and environmental sources. Environ. Mutagen. 6: 651-668.

Casto, B. C.; Hatch, G. G.; Huang, S. L.; Huisingh, J. L.; Nesnow, S.; Waters, M. D. (1981) Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: in vitro mutagenesis and oncogenic transformation. Environ. Int. 5: 403-409.

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

Claxton, L. D. (1983) Characterization of automotive emissions by bacterial mutagenesis bioassay: a review. Environ. Mutagen. 5: 609-631.

Claxton, L.; Kohan, M. (1981) Bacterial mutagenesis and the evaluation of mobile-source emissions. In: Waters, M. D.; Sandhu, S. S.; Huisingh, J. L.; Claxton, L.; Nesnow, S., eds. Short-term bioassays in the analysis of complex environmental mixtures II: proceedings of the second symposium on the application of short-term bioassays in the fractionation and analysis of complex environmental mixtures; March 1980; Williamsburg, VA. New York, NY: Plenum Press; pp. 299-317. (Hollaender, A.; Welch, B. L.; Probstein, R. F., eds. Environmental science research series: v. 22).

Curren, R. D.; Kouri, R. E.; Kim, D. M.; Schectman, L. M. (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.

Dukovich, M.; Yasbin, R. E.; Lestz, S. S.; Risby, T. H.; Zweidinger, R. B. (1981) The mutagenic and SOS-inducing potential of the soluble organic fraction collected from diesel particulate emissions. Environ. Mutagen. 3: 253-264.

Eddy, E. P.; McCoy, E. C.; Rosenkranz, H. S.; Mermelstein, R. (1986) Dichotomy in the mutagenicity and genotoxicity of nitropyrenes: apparent effect of the number of electrons involved in nitroreduction. Mutat. Res. 161: 109-111.

Federal Register. (1986) Guidelines for mutagenicity risk assessment. F. R. (September 24) 51: 34006-34012.

Guererro, R. R.; Rounds, D. E.; Orthoefer, J. (1981) Sister chromatid exchange analysis of Syrian hamster lung cells treated in vivo with diesel exhaust particulates. Environ. Int. 5: 445-454.

Hinners, R. G.; Burkart, J. K.; Malanchuk, M. (1980) Facilities for diesel exhaust studies. In: Pepelko, W. E.; Danner, R. M.; Clarke, N. A., eds. Health effects of diesel engine emissions: proceedings of an international symposium; December 1979. Cincinnati, OH: U.S. Environmental Protection Agency, Health Effects Research Laboratory; pp. 681-697; EPA report no. EPA-600/9-80-057b. Available from: NTIS, Springfield, VA; PB81-173817.

Huisingh, J.; Bradow, R.; Jungers, R.; Claxton, L.; Zweidinger, R.; Tejada, S.; Bumgarner, J.; Duffield, F.; Waters, M.; Simmon, V. F.; Hare, C.; Rodriguez, C.; Snow, L. (1978) Application of bioassay to the characterization of diesel particle emissions. In: Waters, M. D.; Nesnow, S.; Huisingh, J. L.; Sandhu, S. S.; Claxton, L., eds. Application of short-term bioassays in the fractionation and analysis of complex environmental mixtures: [proceedings of a symposium; February; Williamsburg, VA]. New York, NY: Plenum Press; pp. 383-418. (Hollaender, A.; Probstein, F.; Welch, B. L., eds. Environmental science research: v. 15).

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

Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. (1986) Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V. (Developments in toxicology and environmental science: v. 13).

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

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

Li, A. P.; Dutcher, J. S. (1983) Mutagenicity of mono-, di-, and tri-nitropyrenes in Chinese hamster ovary cells. Mutat. Res. 119: 387-392.

Li, A. P.; Royer, R. E. (1982) Diesel-exhaust-particle extract enhancement of chemical-induced mutagenesis in cultured Chinese hamster ovary cells: possible interaction of diesel exhaust with environmental chemicals. Mutat. Res. 103: 349-355.

Liber, H. L.; Andon, B. M.; Hites, R. A.; Thilly, W. G. (1981) Diesel soot: mutation measurements in bacterial and human cells. Environ. Int. 5: 281-284.

Liberti, A.; Ciccioli, P.; Cecinato, A.; Brancaleoni, E.; Di Palo, C. (1984) Determination of nitrated-polyaromatic hydrocarbons (nitro-PAHs) in environmental samples by high resolution chromatographic techniques. J. High Resolut. Chromatogr. Chromatogr. Commun. 7: 389-397.

Matsushita, H.; Goto, S.; Endo, O.; Lee, J.-H.; Kawai, A. (1986) Mutagenicity of diesel exhaust and related chemicals. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 103-118. (Developments on toxicology and environmental science: v. 13).

Mitchell, A. D.; Evans, E. L.; Jotz, M. M.; Riccio, E. S.; Mortelmans, K. E.; Simmon, V. F. (1981) Mutagenic and carcinogenic potency of extracts of diesel and related environmental emissions: in vitro mutagenesis and DNA damage. Environ. Int. 5: 393-401.

Morimoto, K.; Kitamura, M.; Kondo, H.; Koizumi, A. (1986) Genotoxicity of diesel exhaust emissions in a battery of in-vitro short-term bioassays. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 85-102. (Developments in toxicology and environmental science: v. 13).

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.

Nishioka, M. G.; Petersen, B. A.; Lewtas, J. (1982) Comparison of nitro-aromatic content and direct-acting mutagenicity of diesel emissions. In: Cooke, M.; Dennis, A. J.; Fisher, G. L., eds. Polynuclear aromatic hydrocarbons: physical and biological chemistry. Columbus, OH: Battelle Press; pp. 603-613.

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

Pepelko, W. E.; Danner, R. M.; Clarke, N. A., eds. (1980) Health effects of diesel engine emissions: proceedings of an international symposium, v. 2; December 1979; Cincinnati, OH. Cincinnati, OH: U.S. Environmental Protection Agency, Health Effects Research Laboratory; EPA report no. EPA-600/9-80-057b. Available from: NTIS, Springfield, VA; PB81-173817.

Pereira, M. A.; Connor, T. H.; Meyne, J.; Legator, M. S. (1981a) Metaphase analysis, micronucleus assay and urinary mutagenicity assay of mice exposed to diesel emissions. Environ. Int. 5: 435-438.

Pereira, M. A.; Sabharwal, P. S.; Gordon, L.; Wyrobek, A. J. (1981b) The effect of diesel exhaust on sperm-shape abnormalities in mice. Environ. Int. 5: 459-460.

Pereira, M. A.; Sabharwal, P. S.; Kaur, P.; Ross, C. B.; Choi, A.; Dixon, T. (1981c) *In vivo* detection of mutagenic effects of diesel exhaust by short-term mammalian bioassays. Environ. Int. 5: 439-443.

Pereira, M. A.; McMillan, L.; Kaur, P.; Gulati, D. K.; Sabharwal, P. S. (1982) Effect of diesel exhaust emissions, particulates, and extract on sister chromatid exchange in transplacentally exposed fetal hamster liver. Environ. Mutagen. 4: 215-220.

Russell, L. B.; Generoso, W. M.; Oakberg, E. F.; Russell, W. L.; Bangham, J. W.; Stelzner, K. F. (1980) Tests for heritable effects induced by diesel exhaust in the mouse. Martin Marietta Energy Systems, Inc., Oak Ridge National Laboratory; report no. ORNL-5685.

Salmeen, I.; Durisin, A. M.; Prater, T. J.; Riley, T.; Schuetzle, D. (1982) Contribution of 1-nitropyrene to direct-acting Ames assay mutagenicities of diesel particulate extracts. Mutat. Res. 104: 17-23.

Schuetzle, D.; Frazier, J. A. (1986) Factors influencing the emission of vapor and particulate phase components from diesel engines. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 41-63. (Developments in toxicology and environmental science: v. 13).

Schuetzle, D.; Lewtas, J. (1986) Bioassay-directed chemical analysis in environmental research. Anal. Chem. 58: 1060A-1076A.

Schuetzle, D.; Perez, J. M. (1983) Factors influencing the emissions of nitrated-polynuclear aromatic hydrocarbons (nitro-PAH) from diesel engines. J. Air Pollut. Control Assoc. 33: 751-755.

Schuler, R. L.; Niemeier, R. W. (1981) A study of diesel emissions on Drosophila. Environ. Int. 5: 431-434.

Shelburne, J. D.; Chescheir, G. M., III; Garrett, N. E.; Huisingh, J. L.; Waters, M. D. (1981) Mutagenic effects of environmental particulates in the CHO/HGPRT system. In: Waters, M. D.; Sandhu, S. S.; Huisingh, J. L.; Claxton, L.; Nesnow, S., eds. Short-term bioassays in the analysis of complex environmental mixtures II: proceedings of the second symposium on the application of short-term bioassays in the fractionation and analysis of complex environmental mixtures; March 1980; Williamsburg, VA. New York, NY: Plenum Press; pp. 337-350. (Hollaender, A.; Welch, B. L.; Probstein, R. F., eds. Environmental science research series: v. 22).

Siak, J. S.; Chan, T. L.; Lees, P. S. (1981) Diesel particulate extracts in bacterial test systems. Environ. Int. 5: 243-248.

Tucker, J. D.; Xu, J.; Stewart, J.; Baciu, P. C.; Ong, T.-m. (1986) Detection of sister chromatid exchanges induced by volatile genotoxicants. Teratog. Carcinog. Mutagen. 6: 15-21.

# 10. METABOLISM AND MECHANISM OF ACTION IN DIESEL EMISSION-INDUCED CARCINOGENESIS

Considerable research effort has been directed toward assessing the carcinogenic potential of diesel engine emissions. As indicated in Chapter 7, whole diesel exhaust is a pulmonary carcinogen in rats exposed chronically to high concentrations, but the mechanism lof carcinogenicity remains uncertain. The involvement of genetic or epigenetic mechanisms, or a combination of these, remains to be determined regarding the pulmonary carcinogenicity of diesel exhaust. That a definitive mechanism of action has eluded investigators is not surprising when one considers that diesel exhaust is a complex mixture of hundreds of chemicals and soot particles. In examining possible mechanisms of action of diesel exhaust-induced carcinogenicity, it is necessary to address the following major areas: (1) the metabolism and mechanism of action of known carcinogenic components such as benzo[*a*]pyrene (B[*a*]P) and various nitroarenes, (2) the carcinogenic potential of the soot particle, (3) the role of pulmonary leukocytes in the development of lung tumors, and (4) the molecular dosimetry of inhaled diesel exhaust.

## 10.1 METABOLISM AND MECHANISM OF ACTION OF ORGANIC CARCINOGENIC COMPONENTS OF DIESEL EXHAUST

The metabolism and proposed carcinogenic mechanism of action of chemicals are often interrelated. It is difficult to obtain an understanding of one without consideration of the other and, therefore, both of these processes are being considered in this discussion. Specifically, emphasis will be given to polycyclic aromatic hydrocarbons (PAHs) and nitroarenes. These components are of greatest concern because of their demonstrated or suspected activity as procarcinogens or carcinogens in laboratory animals and their universal occurrence in diesel emissions. It is also well known that PAH biological reactivity and carcinogenicity are dependent on their metabolic conversion [reviewed by Conney (1982)].

The mechanism of action of many PAH carcinogens has been attributed to the reactivity of certain metabolic intermediates with cellular macromolecules and the subsequent formation

of DNA adducts. The organics adsorbed to diesel exhaust particles may become available for biotransformation to known reactive intermediates, and macromolecular binding of these metabolites has been demonstrated.

Except for some of the DNA adduct studies, the available data base does not allow for a definitive discussion of the specific mechanism of carcinogenic action for these compounds relative to diesel exhaust specifically but rather is approached from the standpoint of the chemicals per se. Some of the data are derived primarily from in vitro studies that were not specifically concerned with the potential carcinogenicity of diesel exhaust but are relevant because the compounds of concern are known components of diesel emissions.

Several long-term inhalation studies have provided evidence for carcinogenicity and tumorigenicity of whole diesel exhaust in animals (Heinrich et al., 1986; Iwai et al., 1986; Mauderly et al., 1987). Over 100 carcinogenic or potentially carcinogenic components have been specifically identified in diesel emissions, including various PAHs and nitroarenes such as 1-nitropyrene (1-NP) and dinitropyrenes (DNPs). These compounds are adsorbed to the carbon core of the particulate phase of the exhaust and, upon desorption, may become available for biological processes such as metabolic activation to mutagens. Among compounds identified from diesel exhaust are B[*a*]P, dibenz[*a,h*]anthracene, pyrene, chrysene, and nitroarenes such as 1-NP, 1,3-DNP, 1,6-DNP, and 1,8-DNP, all of which are mutagenic, carcinogenic, or implicated as procarcinogens or cocarcinogens (Stenback et al., 1976; Weinstein and Troll, 1977; Thyssen et al., 1981; Pott and Stöber, 1983; Howard et al., 1983; Hirose et al., 1984; Nesnow et al., 1984; El-Bayoumy et al., 1988).

### 10.1.1 Metabolism and Disposition of Benzo[*a*]pyrene

It is generally recognized that B[*a*]P is an activation-dependent carcinogen, with the activated metabolites forming covalent DNA adducts (Boyland, 1980). The reactions responsible for this activation are mediated by the cytochrome P-450 monooxygenases and are known to occur in multiple tissues and in different species. The activation proceeds through Phase I oxidative and hydrolytic reactions, which result in the formation of the ultimate carcinogenic metabolite, B[*a*]P 7,8-dihydrodiol 9,10-epoxide. Specifically, B[*a*]P undergoes a mixed function oxidase (MFO)-mediated epoxidation to form B[*a*]P-7,8-oxide, which, in turn, is subjected to an epoxide hydrolase-mediated hydrolysis resulting in the

stereoisomeric diols, (+)-B[*a*]P 7,8-dihydrodiol, and (-)-B[*a*]P 7,8-dihydrodiol. The diasterioisomeric forms of B[*a*]P 7,8-diol 9,10-epoxide are derived following another P-450-mediated reaction.

The principal source of environmental B[*a*]P is its association with airborne particles such as those generated by diesel engines and coke ovens (U.S. Environmental Protection Agency, 1985). Therefore, understanding the metabolism of chemical carcinogens such as B[*a*]P is instrumental in providing a complete understanding of diesel emission-induced carcinogenesis. However, it is beyond the scope of this document to review exhaustively the literature regarding the metabolism of these compounds. The respiratory tract metabolism of particle-associated PAHs has been summarized by Sun et al. (1988a)

Relative to diesel exhaust carcinogenicity, several studies have examined the metabolism and disposition of constituents such as B[*a*]P. Mitchell (1982), subjected 24 male F344 rats to nose-only inhalation of $^{3}$H-B[*a*]P aerosol (500 mg/m$^{3}$) for 60 min. High levels of radiolabel were detected in the trachea, lungs, and turbinates. Based on measurement of the radiolabel, biphasic clearance was noted with half-time ($t_{1/2}$) values of 2 to 3 h and 25 to 56 h. Absorption by the lungs and systemic distribution was demonstrated by the presence of radiolabel in soft tissues, such as the liver, kidney, gastrointestinal tract, spleen, brain, and testes. The majority of the radiolabel in these tissues was removed after 2 days, and the major route of excretion was in the feces. The significance of this study is the demonstration of rapid absorption and systemic distribution of B[*a*]P and potential metabolites following inhalation exposure.

Metabolism of intratracheally instilled B[*a*]P (1.0 $\mu$g) in strain A/J mice exposed to diluted diesel exhaust (8 h/day, 7 days/week for 9 mo) was reported by Tyrer et al. (1981). The radiolabel ($^{14}$C or $^{3}$H) was rapidly distributed throughout the body within 2 h. The highest levels were detected in the lungs, liver, and gastrointestinal tract. Only trace levels were detected in the gastrointestinal tract 168 h after administration. A companion study (Cantrell et al., 1980) examined the effects of prior diesel exhaust exposure on in vivo B[*a*]P metabolism in the aforementioned mice. Homogenates of lung, liver, and testes were obtained from five mice sacrificed at 2, 24, or 168 h after B[*a*]P instillation. High-performance liquid chromatography (HPLC) analysis detected free B[*a*]P and nonconjugated primary metabolites, and sulfate, glucuronide and glutathione conjugates in

each of the tissues. The occurrence of primary and secondary B[*a*]P metabolites in all three tissues was verified. The major hepatic metabolite was 3-hydroxy-B[*a*]P. The investigators concluded that the diesel exhaust exposure may qualitatively affect the metabolism of B[*a*]P but does not significantly affect the distribution of B[*a*]P.

Sun et al. (1984) provided additional information comparing the disposition of particle-adsorbed B[*a*]P (0.1 wt %) and pure B[*a*]P following 30-min nose-only inhalation by F344 rats. Long-term lung retention (percentage retained after 7 days) of particle-adsorbed $^{3}$H-B[*a*]P was approximately 230-fold greater than that for pure $^{3}$H-B[*a*]P. Pulmonary clearance of particle-associated $^{3}$H was biphasic, with an initial $t_{1/2}$ of 1 h and a second-phase $t_{1/2}$ of 18 days, the latter representing clearance of 50% of the initially deposited radiolabel. Clearance of pure B[*a*]P aerosol was >99% within 2 h and was apparently caused by pulmonary and mucous membrane absorption into the blood rather than by mucociliary clearance and subsequent ingestion (Sun et al., 1982). Of the radiolabel retained in the lungs, 65 to 76% was B[*a*]P, 13 to 17% was B[*a*]P-phenol, and 5 to 18% was B[*a*]P-quinone. Although the Sun et al. (1984) study demonstrated the biotransformation of B[*a*]P to several metabolites, the epoxide intermediates known to be carcinogenic (Sims et al., 1974; Slaga et al., 1976) were not identified. However, B[*a*]P-phenol metabolites are reported to be mutagenic (Glatt and Oesch, 1976; Wislocki et al., 1986; Wood et al., 1976).

Leung et al. (1988) studied the role of microsomes in the removal and metabolism of B[*a*]P from diesel exhaust particles. Hepatic and lung microsomal preparations were made from 3-methylcholanthrene-induced F344 rats. $^{14}$Carbon-B[*a*]P was adsorbed to diesel exhaust particles (0.49 $\mu$Ci/mg) and incubated with the microsomal preparations. Results indicated that both lung and liver microsomes were capable of removing B[*a*]P from the exhaust particles and that this capacity was dependent on the lipid content of the microsomes. Only small (<3%) amounts of B[*a*]P were transferred from the particles, with only 1 to 2% of this being metabolized. Free B[*a*]P, however, was extensively metabolized by the microsomes to B[*a*]P-9-10-diol. Relative to the liver microsomes, the lung microsomes exhibited an approximate twofold greater efficiency in the transfer of particle-associated B[*a*]P.

Bond et al. (1984) demonstrated metabolism of particle-associated B[*a*]P and free B[*a*]P by alveolar macrophages (AMs). B[*a*]P-9,10-diol and B[*a*]P-7,8-diol were identified in the

culture media, and B[*a*]P-7,8-diol and B[*a*]P-4,5-diol were detected in the cellular extracts. Additionally, small amounts of B[*a*]P phenols and B[*a*]P quinones were detected in both the cells and the media. The total amount of metabolites from both the cells and media were increased with increasing incubation time up to 48 h. However, use of B[*a*]P in solution or B[*a*]P coated onto diesel exhaust particles did not alter the total amount of metabolites produced by the macrophages over a 24-h incubation period. Alveolar macrophage-mediated metabolism of particle-associated B[*a*]P is especially relevant considering that macrophages are instrumental in sequestering and transporting diesel exhaust particulate matter in the lungs. Although this investigation points to the ability of the AMs to metabolize B[*a*]P associated with diesel particles, Chen and Vostal (1982) have reported that aryl hydrocarbon hydroxylase (AHH) in AMs is decreased after in vivo exposure to diesel exhaust. Whether such diesel-associated decreases in AM enzymatic activity is counterbalanced by increases in the AM population size in response to diesel particle deposition (White and Garg, 1981) is unknown. Although it is known that human AMs contain AHH activity (McLemore et al., 1981), and that they can metabolize B[*a*]P (Harris, 1985), comparative studies of the AHH activities in rat, hamster, and human AMs could contribute toward determining the relationship such activity may have on the development of lung tumors.

Even though the AMs appear to contain the bulk of diesel particles deposited in the lung during chronic exposures, other cell types may also participate in the sequestration and/or metabolic activation of carcinogenic agents. The ability of lung epithelial cells to sequester diesel exhaust particles was reported by White and Garg (1981). Furthermore, significant metabolism of B[*a*]P by rat Type II alveolar epithelial cells was reported by Bond et al. (1983). In this study, a lung epithelial cell line (LEC) was shown to metabolize B[*a*]P to B[*a*]P-7,8-diol and B[*a*]P-9,10-diol, the latter accounting for 80% of the total B[*a*]P metabolites. Small quantities of glucuronide conjugates of B[*a*]P-7,8-diol and 9-hydroxy-B[*a*]P were detected. Preexposure of the cells to diesel exhaust particle extract, benz[*a*]anthracene, or coal gas condensate increased rates of covalent binding of radioactivity to macromolecules twofold to fivefold. It was also found that pretreatment of LEC with diesel exhaust particle extract produced a threefold increase in covalent binding of $^{14}$C-B[*a*]P. Compared with the AMs that were examined in the aforementioned study, the rat Type II cells showed approximately a ten times greater ability to metabolize B[*a*]P.

Under healthy conditions, the Type II cells represent about 12 to 16% of all cells in the pulmonary epithelium of mammalian lungs and account for approximately 4 to 9% of the cells in the lungs (Crapo et al., 1983). Alveolar macrophages, on the other hand, account for approximately 4 to 9% of the cells in the pulmonary region (Crapo et al., 1983). In terms of their relative availability and existing information on their relative abilities to metabolize B[*a*]P, the Type II cells may play an even more important role in metabolically activating PAH than the AMs, assuming PAH as a substrate is available to them (e.g., extraction of PAH from diesel particles by AMs and the subsequent release of PAH or metabolically susceptible metabolites of PAH at Type II cell sites). The Type II cell hyperplasia observed after the deposition of diesel and other types of particles (White and Garg, 1981; Lee et al., 1986; Lee et al., 1988; Plopper et al., 1983) seemingly would favor a prominent role for these cells in producing activated PAH metabolites. Another cell type that may be important in the metabolism of PAH to ultimate carcinogens is the nonciliated bronchiolar cell. These cells are relatively rich in chemical metabolizing enzymes and, being also in a region of the respiratory tract where clearance of material would be relatively fast, may receive exposure via mucus to organics that have desorbed in the pulmonary region. The respiratory tract cytochrome P-450 system, for example, is present in Type II cells but it is not as concentrated in this epithelial cell type as it is in the nonciliated bronchiolar cell (Boyd, 1984). It is worthy to note that bronchoalveolar adenomas that develop following diesel exposure have been found to resemble both Type II and nonciliated bronchiolar cells (Mauderly et al., 1987). Like the Type II cells, the nonciliated bronchiolar cells are viewed as not being important in the phagocytosis of particles that deposit in the lung. As previously indicated, any metabolism of procarcinogens by these cells probably involves the preextraction of carcinogen(s) in the extracellular lining fluid and/or in other endocytic cells.

It is evident from the preceding studies that B[*a*]P adsorbed to diesel exhaust particles that are deposited in the respiratory tract can be readily distributed throughout much of the organism via absorption from the lung and transport by the mucociliary escalator to the gastrointestinal tract. The current data base appears to support the contention that particle-associated B[*a*]P can ultimately be metabolized by AMs and/or Type II cells to reactive intermediates following deep lung deposition of these particles.

### 10.1.2 Carcinogenic Mechanism of Benzo[*a*]pyrene

As a result of PAH metabolism studies such as those conducted on B[*a*]P, theories have been proposed regarding the molecular mechanism by which activated intermediates express their genotoxic effects. Benzo[*a*]pyrene served as the model for the "bay-region" concept summarized by Jerina et al. (1980). This proposed mechanism would also be applicable to such compounds as benz[*a,h*]anthracene, which is a potent carcinogen also known to occur as a diesel exhaust combustion product.

Briefly, this concept states that compounds derived from an angular benz[*a*]anthracene nucleus may undergo epoxidation, and if the resulting epoxides are located in the bay region, they will be better alkylating agents and, therefore, have a greater genotoxic potential. The chemical reactivity of these bay-region epoxides is positively correlated with biological reactivity of these compounds.

Based on the assumption that DNA adduct formation is a critical step in the initiation of carcinogenesis (Harris, 1985), increased residence time of PAHs in the lung would increase the opportunity for metabolism and subsequent adduct formation. This would be especially important if association of the PAHs with the soot particles and their slow release from these particles contributed to this increased residence time. Therefore, comparison of adduct formation by B[*a*]P alone to that of particle-associated B[*a*]P is important for understanding possible mechanisms of diesel exhaust carcinogenicity.

An experiment was undertaken to test the hypothesis that inhalation of B[*a*]P associated with carbon black (CB) particles would increase the levels of DNA adducts compared with inhalation of pure B[*a*]P (Wolff et al., 1989). The DNA modification was measured using the $^{32}$P-postlabeling method recently developed by Randerath et al. (1985). The high sensitivity ($\approx$1 adduct in $10^{10}$ bases) of this technique (Reddy and Randerath, 1986) made possible measurement of the low levels of DNA adducts resulting from repeated inhalation exposures to $^{14}$C-B[*a*]P aerosols (2 mg/m$^3$), $^{14}$C-B[*a*]P (2 mg/m$^3$) adsorbed to CB particles (97 mg/m$^3$) (B[*a*]P/CB), or filtered air. Total $^{14}$C levels in the lung (a nonspecific indicator of reactive and nonreactive B[*a*]P metabolites, free B[*a*]P, and particle bound B[*a*]P) were 100-fold greater following exposure to B[*a*]P/CB than following exposure to B[*a*]P alone.

The levels of total DNA adducts or the B[*a*]P diol-epoxide(BPDE)-DNA adduct in the lung were *not* significantly different whether the rats were exposed to pure B[*a*]P or

B[*a*]P/CB. However, association of B[*a*]P with CB resulted in the formation of unidentified lung adducts that were not seen in DNA from lungs of rats exposed to pure B[*a*]P. It is possible that the adducts seen only in the B[*a*]P/CB exposures may play a role in the potential tumorigenic effect of particle-associated B[*a*]P. Reasons for the discrepancy between particle effects on total DNA adducts and retention of $^{14}C$ include the possibility that the kinetics for formation and decline of DNA adducts are different from those of total bound $^{14}C$. As a consequence, long-term retention of total B[*a*]P and metabolites in the lung may not be a good marker for adduct formation.

There were clear differences in the kinetics of the buildup and decline of DNA adduct levels and total $^{14}C$ for rats exposed to B[*a*]P/CB. The $t_{1/2}$ for the decline of total $^{14}C$ was approximately tenfold faster than that for the decline in levels of DNA adducts for rats exposed to B[*a*]P/CB. Previous work has shown that at 1 day or later after the end of single exposures to B[*a*]P or B[*a*]P/CB, most of the $^{14}C$ present was bound to total macromolecules (Sun et al., 1988b), presumably largely, non-DNA protein. Thus, this information in combination with the current data suggests that decline or repair of DNA adducts is considerably faster than that of protein turnover. Following repeated exposures, this would be expected to lead to increased buildup of $^{14}C$ in the lung relative to DNA adducts. The $t_{1/2}$ values for decline in DNA adducts observed in the current work are similar to the $t_{1/2}$ values of approximately 4 weeks reported for B[*a*]P metabolite-DNA adducts in the lungs of A/HeJ and C57Bl/6J mice (Stowers and Anderson, 1985). Protein turnover is generally on longer time scales than the aforementioned $t_{1/2}$ values.

It appears that long-term retention of $^{14}C$ radiolabel in the lung may not be as important as previously suspected, at least with respect to indicating DNA damage. The $^{14}C$ binding levels and DNA adducts were not closely related, and it is clear from these results that DNA adduct levels cannot be predicted from total $^{14}C$ levels. This observation is consistent with the work of Morse and Carlson (1985) who observed that binding levels of $^{3}H$ with lung protein were greater than levels of $^{3}H$ to lung DNA 6 h after administration of oral H-B[*a*]P to mice. They also found that $^{3}H$ binding to protein was more persistent than $^{3}H$ binding to DNA.

Caution should be used in interpreting the results from short-term exposures in regard to possible implications for long-term exposures when carcinogenicity might be observed.

The same pattern of results seen after 12 weeks might not continue after many months of exposure. The adduct levels were higher in the rats exposed to B[*a*]P/CB than B[*a*]P after 12 weeks of exposure, and so it is possible that this difference might become greater with continued exposure. In addition, the different adduct patterns between the B[*a*]P/CB and B[*a*]P exposures may indicate that other adducts besides the BPDE-DNA adduct are important in potential carcinogenic effects of B[*a*]P/CB exposures. Another factor to consider is the possible influence of a chronic inflammatory response, cell injury, and cell proliferation, which accompany long-term exposures to inhaled insoluble particles (Morrow, 1986). Such responses are generally greater after prolonged exposure than those in the current 12-week exposure. These responses might be factors in progression to tumors in long-term inhalation exposures of rodents, when large lung burdens of particles accumulate (Morrow, 1986), and in the increased incidence in tumors, when B[*a*]P is merely mixed with $Fe_2O_3$ particles versus adsorbed onto the particle (Saffiotti et al., 1965).

### 10.1.3 Metabolism and Disposition of Nitroarenes

Diesel engine emissions contain a large number of components including an extensive list of nitroarenes. Quantitatively, the nitroarenes represent a relatively small contribution to the overall PAH component of diesel engine emissions. However, with respect to the carcinogenic potential of diesel exhaust, some of the nitroarenes (e.g., 1-NP, 4-NP, 6-nitrochrysene, and some DNPs) are of concern because of their known or suspected carcinogenic activity and their high mutagenic activity in some test systems (Manabe et al., 1985; International Agency for Research on Cancer, 1989). Within the scope of this document, it is inappropriate to review of all of the studies regarding the carcinogenicity, metabolism, and mechanism of action of these various nitroarenes. Therefore, emphasis has been placed on those nitroarenes considered by the International Agency for Research on Cancer (1989).

1-Nitropyrene, a genotoxic and carcinogenic nitro-substituted organic, is a particle-associated component of diesel exhaust (Pitts et al., 1982; Schuetzle et al., 1982; King, 1988). As with B[*a*]P, several investigators have studied the metabolism and disposition of 1-NP both in free form and in association with diesel exhaust particles.

Bond and Mauderly (1984) made quantitative measurements of 1-NP metabolism and macromolecular covalent binding in the isolated-perfused rat lung. The study verified oxidation, reduction, acetylation, and conjugation biotransformation of 1-NP by the lung, with oxidation being the major process. The major metabolites were 3-, 6-, and 8-hydroxynitropyrene. The overall metabolism of 1-NP was increased by prior exposure of the rats to the mixed-function oxygenase (MFO) inducer 3-methylcholanthrene (3-MC) but not to phenobarbital. This 3-MC-induced increase in 1-NP metabolism and a parallel increase in macromolecular covalent binding suggests that this pathway may be responsible for the observed covalent binding.

It is also noteworthy that hydroxynitropyrenes have been shown to be mutagenic using the Ames assay (El-Bayoumy and Hecht, 1983).

Exposure of rats to diesel exhaust (7.4 mg/m$^3$) for 7 h/day, 5 days/week for 4 weeks resulted in twofold increases in the rates of nitropyrene metabolism in nasal tissue and in isolated perfused lungs from these animals (Bond et al., 1986). High-performance liquid chromatography analysis of ethyl acetate-extractable 1-[$^{14}$C]NP metabolites indicated that the major metabolites were 3-, 6-, and 8-hydroxy-1-aminopyrene and 4,5-dihydro-4,5-dihydroxy-1-nitropyrene. Furthermore, a fourfold increase in $^{14}$C covalently bound in the lungs of these rats was detected. The increase in 1-NP metabolism was not observed for rats of lower-exposure (0.35 or 3.3 mg/m$^3$) groups or clean air controls. The data from this study indicate that exposure to diesel exhaust particulate matter at concentrations of 7.4 mg/m$^3$ significantly alters the metabolism and subsequent covalent binding of nitropyrene.

Bond et al. (1986) also examined the metabolism and deposition of free and particle-associated 1-NP in F344 rats. Results of the work indicated that the urinary and fecal excretion of $^{14}$C-1-NP was not altered by exposure to the pure form or to that adsorbed on diesel exhaust particles. Pure 1-NP was more efficiently absorbed in the lung than was 1-NP coated onto diesel exhaust particles, and, therefore, greater lung retention was noted for particle-adsorbed 1-NP. However, no significant difference between the two forms of 1-NP was noted for extrapulmonary tissue distribution or metabolic profiles. Analysis of excreta and tissues indicated that 1-NP is rapidly metabolized by the lungs or metabolized by other tissues following translocation from the lungs. For both 1-NP forms, small amounts of 6- and 8- hydroxyacetylaminopyrene were detected in the lungs, suggesting pulmonary

oxidation, reduction, and conjugation of the parent compound. The demonstration of pulmonary metabolism of 1-NP and greater retention of 1-NP when adsorbed to diesel exhaust particles may be significant relative to the dose to the lungs of both parent compound and metabolites.

Ball and King (1985) administered [$^{14}$C]1-NP to rats intraperitoneally, orally, or by intratracheal instillation of vapor-phase-coated diesel exhaust particles (380 $\mu$g [$^{14}$C]1-NP/g; 5 mg/rat). Over 50% of the radiolabel was recovered (20 to 30% in the urine and 40 to 60% in the feces) within 24 h, regardless of the route of administration. The metabolic profile and elimination kinetics were similar for all routes of administration. The principle urinary metabolite (representing 15 to 25% of the total urinary $^{14}$C) was 6-hydroxy-*N*-acetyl-1-aminopyrene (6-OH-NAAP), a compound with demonstrated S-9 dependent mutagenic activity in *Salmonella* strain TA98. Gut flora was shown to be necessary for the formation of 6-OH-NAAP, for the observed enterohepatic circulation of metabolites excreted in the bile, and for excretion of mutagenic activity in the urine. That intestinal microorganisms may alter the metabolites of 1-NP and facilitate their reabsorption was also reported by Medinsky et al. (1985). Accumulation of $^{14}$C and diesel exhaust particles was detected in the lungs and gastrointestinal tract 24 h after intratracheal administration, thereby attesting to the importance of mucociliary transport and distribution of particles and their adsorbed components. Based on these results and previous in vitro studies (King et al., 1983) demonstrating 1-NP binding to macromolecules, the authors note the possible risk to the gastrointestinal tract and lungs relative to 1-NP.

The biotransformation of 1-NP by intestinal microflora of humans, rats, and mice was also reported by King et al. (1990). Metabolites including 1-aminopyrene, *N*-acetyl-1-aminopyrene, *N*-formyl-1-aminopyrene, and two unkown compounds were detected. Except for *N*-formyl-1-aminopyrene, all of the metabolites as well as the residual parent compound were mutagenic in reverse-mutation assays.

Medinsky et al. (1988) examined the distribution of covalently bound $^{14}$C following the administration of [$^{14}$C]-1-NP to rats by nose-only inhalation (340 ng/L for 1 h) or gavage (4.2 $\mu$g in 0.5 mL saline). Ninety-six hours after administration, covalently bound $^{14}$C was highest in the kidney, followed by the liver and lungs, respectively. These findings suggest

that the kidney may be a potential target organ for 1-NP, whether the route of administration is inhalation or gavage.

Howard et al. (1986) studied the binding of intratracheally instilled nitropyrenes and B[*a*]P to mouse lung DNA following preexposure to intratracheally instilled doses of the putative inducing agents, B[*a*]P, dichloromethane extract of diesel exhaust, or 1-NP. The results indicated that 1-NP was a potent DNA-binding agent even in the absence of enzyme induction and that this potency was increased following B[*a*]P exposure. Dinitropyrene (a mixture of the 1,3-, 1,6-, and 1,8- isomers) was also a potent lung DNA-binding agent, with and without the inducers. Benzo[*a*]pyrene was not as potent a binding agent. Preexposure to the diesel exhaust extract, but not to B[*a*]P, resulted in increased DNA binding of B[*a*]P. Pretreatment with the dichloromethane extract of diesel exhaust failed to increase the DNA binding of the nitropyrenes. The significance of this report is the demonstration that exposure to diesel exhaust may potentiate the DNA binding of some of its components.

King (1988) provided information relating the metabolism of nitropyrenes and their carcinogenic potential. Briefly, DNPs were found to be much more carcinogenic than 1-NP. The cytosolic enzymes of the rat mammary gland activated DNPs by monoreduction to hydroxylamine followed by O-acetylation. Reduction and acetylation pathways for DNPs and subsequent DNA adduct formation were detected in intact cells. However, the intact mammary cells metabolized 1-NP primarily through oxidative pathways.

Physiologically based pharmacokinetic modeling was used by Medinsky and co-workers to simulate the disposition of 1-NP after its inhalation or ingestion by rats (Medinsky et al., 1989). The model utilized physiologically realistic organ volumes, organ blood flow values, and tissue/blood partition coefficients for 1-NP and its metabolites. 1-Nitropyrene displayed a higher affinity for blood than for lung tissue (lung/blood partition coefficient of 0.5), whereas the liver/blood and kidney/blood partition coefficients were equal to 1, implying that perfusion rate alone could account for removal of 1-NP from these organs. The partition coefficients for the 1-NP metabolites were less than that of 1-NP, indicating that these would more efficiently partition into the blood and be excreted via the urine or bile. These findings are consistent with the reported rapid excretion of 1-NP metabolites.

The International Agency for Research on Cancer (1989) classifies 4-nitropyrene (4-NP) as a possible human carcinogen and identifies it as a component of diesel exhaust. However, little information is available regarding the metabolism of this compound. One report (Fu et al., 1986) reviewed in International Agency for Research on Cancer (1989) reported the in vitro biotransformation of 4-NP to 4-nitropyrene 9,10-dione, 8-hydroxy-4-nitropyrene-1,6-hydroquinone by rat liver microsomes. The report also indicated that this metabolite was mutagenic.

6-Nitrochrysene has been shown to be metabolized to 6-nitrosochrysene, 6-aminochrysene, *N*-formyl-6-aminochrysene, and 6-acetylaminochrysene by anaerobic bacteria isolated from human feces, which implies such metabolites may be formed in the lower gastrointestinal tract (El-Bayoumy and Hecht, 1984). Manning et al. (1988) reported the reduction of 6-nitrochrysene to 6-aminochrysene by cultures of intestinal bacteria from rats and mice and pure cultures of anaerobic bacteria. The in vivo metabolism of 6-nitrochrysene in preweanling mice was implied by the detection of a DNA adduct in the lung and liver that corresponded to a DNA adduct found in microsomal incubations containing calf thymus DNA and 6-aminochrysene trans dihydrodiol (Delclos et al., 1988).

The in vitro biotransformation of 1,3-DNP was verified by Djuric et al. (1985), wherein the compound was reduced to 1-amino-3-nitropyrene, 1-nitro-3-nitrosopyrene, and 1,3-diaminopyrene by rat and dog liver cytosol in an argon atmosphere. The addition of acetyl coenzyme A (Co-A) to the liver cytosol incubations resulted in the formation of an additional metabolite, 1-acetylamino-3-nitropyrene. Although intact rat mammary gland cells did not metabolize 1,3-dinitropyrene, in vitro incubations using cytosolic preparations from this type of cell resulted in the formation of 1-amino-3-nitropyrene and 1,3-diaminopyrene (King et al., 1986; Imaida et al., 1988).

Several studies have reported on the in vivo and in vitro metabolism of 1,6-dinitropyrene. Single ip injections of [$^3$H]1,6-DNP (33.6 $\mu$g) in preweanling CD mice resulted in the detection of an N-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene adduct in lung and liver tissue (Delclos et al., 1987b). Following ip administration of [$^3$H]1,6-DNP (200 $\mu$g/kg) to male Sprague-Dawley rats, this same adduct was detected in the urinary bladder, liver, and kidney, and in mammary epithelium (Djuric et al., 1988). In vitro studies have also demonstrated the biotransformation of 1,6-DNP to 1-amino-6-nitropyrene,

1-nitro-6-nitrosopyrene and 1,6-diaminopyrene by rat and dog liver cytosol (Djuric et al., 1985) and also by rat mammary gland cytosol (King et al., 1986).

The International Agency for Research on Cancer (1989) considers 1,8-DNP to be a possible human carcinogen. In vivo and in vitro studies have demonstrated the formation of several metabolites. Following oral administration of 0.3 mg of 1,8-DNP to male CD rats, N,N'-diacetyl-1,8-diaminopyrene, 1,8-diaminopyrene, 1-acetylamino-8-nitropyrene, and 1-amino-8-nitropyrene were detected in the feces (Heflich et al., 1986). Additional, unidentified polar metabolites were also detected. The fact that only 1-amino-8-nitropyrene and the polar metabolites were detected for germ-free rats treated similarly suggests the involvement of intestinal microflora in the in vivo biotransformation of 1,8-DNP. In this same study, incubation of 1,8-DNP with rat or dog liver cytosol preparations in an argon atmosphere produced 1-amino-8-nitropyrene, 1-nitro-8-nitrosopyrene, and 1,8-diaminopyrene. Incubations of human liver cytosol and 1,8-DNP resulted in the production of 1-amino-8-nitropyrene, and rat mammary gland cytosol incubations under anaerobic conditions produced 1-nitro-8-nitrosopyrene, 1-amino-8-nitrosopyrene, and 1,8-diaminopyrene (King et al., 1986; Imaida et al., 1988).

Moller et al. (1988) have reviewed the in vivo metabolism of 2-nitrofluorene, a constituent of diesel exhaust that the International Agency for Research on Cancer (1989) classifies as possibly carcinogenic to humans. The metabolites 2-aminofluorene, 2-acetylaminofluorene, and 2-formylaminofluorene were detected in the urine of rabbits and in the feces of rats following oral administration of 2-nitrofluorene (Tatsumi and Amano, 1987). Studies using [$^{14}$C]2-nitrofluorene administered orally to rats demonstrated the formation of N-, 1-, 3-, 5-, 7-, 8-, and 9-hydroxy-2-acetylaminofluorene. The International Agency for Research on Cancer (1989) noted that N-hydroxy-2-acetylaminofluorine and 9-hydroxy-2-acetylaminofluorene were known to be carcinogenic in animals. Moller et al. (1987) reported that the isolated perfused rat lung metabolizes 2-nitrofluorene to 9-hydroxy-2-nitrofluorene and an unidentified hydroxylated nitrofluorene.

The preceding studies have shown that some of the nitroarenes known to be constituents of diesel exhaust may undergo biotransformation to various metabolites, some of which are known to be carcinogenic to animal species. Such data may become more relevant as a

complete understanding is obtained regarding the desorption of these compounds from the soot particle and their subsequent availability for biotransformation processes.

### 10.1.4 Carcinogenic Mechanism of Nitroarenes

Although the nitroarenes quantitatively represent a relatively small portion of the PAH component of diesel engine exhaust, their contribution to the potential carcinogenicity of diesel engine emissions deserves consideration. In the previous section, information was presented regarding the in vivo and in vitro metabolism of various nitroarenes considered to be possible human carcinogens. The fact that some of these metabolites have been shown to form DNA adducts in animal studies and are mutagenic in several test systems warrants their inclusion in assessing possible mechanisms of diesel-exhaust-induced carcinogenicity.

In a study by Sato et al. (1986), sc injection (4.0 mg total dose) of 1,3-NP, 1,6-NP, or 1,8-NP into male F344 rats resulted in a 100% (10 of 10) sarcoma incidence at the injection site. After 320 days of observation, the dimethyl sulfoxide controls exhibited a tumor incidence of 0% (0 of 20). No tumors were found in rats receiving 4 or 40 mg of 1-NP. Transforming activity was observed for the DNA from 4 of 11 DNP-induced tumors, suggesting oncogene activation.

The absence of tumor formation by 1-NP and the positive results for the DNPs from the previous study are consistent with the findings of King (1988). In this study, female CD and F344 rats were administered 1,3-, 1,6-, or 1,8-DNP, or 1-NP subcutaneously, intraperitoneally, or intragastrically. Based on incidences of malignant histiocytomas, mammary gland tumors and leukemias, the DNPs were much more carcinogenic than 1-NP. Specifically, the potency order was 1,6-DNP > 1,8-DNP > 1,3-DNP > 1-NP. Oral intubation was relatively ineffective in tumor induction by these compounds. Cytosolic enzymes of the rat mammary gland were capable of activating the DNPs to reactive intermediates that formed tRNA adducts. The adduct formation was of the same relative order as the carcinogenic potential and was catalyzed by rat mammary cytosol. In addition to demonstrating the potential of DNPs to induce three tumor types in rats, this study provided data affirming the importance of metabolic activation of the DNPs, the quantitative variability among these isomers relative to their carcinogenic activity, and the susceptibility of a tissue distant from the site of administration to the carcinogenic potential of these

compounds following biotransformation. The data support the conclusion that the DNPs (especially 1,6- and 1,8-NP) are reduced by mammalian enzymes to reactive hydroxylamines, which are in turn activated by acetyl Co-A to potential carcinogens.

Maher et al. (1988) examined the metabolism of 1-NP and DNPs in cultured human fibroblasts. The results of their experiments using these diploid cells indicated that 1-NP underwent bioactivation to a form that produced stable covalent DNA adducts. The mutagenic effect of 1-NP was correlated with the cytotoxic effect, which in turn correlated directly with the number of DNA adducts. It was also reported that 1-nitrosoaminopyrene (1-NOP), a major metabolite of 1-NP was similar to 1-NP in its mutagenic response and extent of DNA adduct formation. To reduce survival of the normal (i.e., not repair-deficient) cells to 37%, 25 1-NP or 1-NOP adducts per $10^6$ DNA nucleotides were required. However, comparisons with repair-deficient cells did indicate that nucleotide excision repair protected against the mutagenic and cytotoxic effects of both 1-NP and 1-NOP. The mutagenic potency of both 1-NP and 1-NOP was intermediate between that of N-acetoxy-2-acetylaminofluorene and B[*a*]P-7,8-diol-9,10-epoxide. Based on *Salmonella typhimurium* assays, Heflich et al. (1986) reported that the mutagenicity of 1-NOP was 20-fold greater than that of 1-NP.

El-Bayoumy et al. (1988) investigated the comparative tumorigenicity of 1-NP and its reduced derivatives, 1-nitrosopyrene and 1-aminopyrene. Results of their tests using Sprague-Dawley rats indicated that 1-NP was considerably more carcinogenic than its reduced derivatives relative to production of mammary adenocarcinomas following administration of these compounds by gavage. These results appear to be in conflict with those of Wislocki et al. (1986) in which 1-nitrosopyrene produced a greater incidence of hepatic tumors in newborn mice.

Roy et al. (1989) suggested that major DNA adducts resulting from the metabolism of 1-NP were not the result of simple nitroreduction. In this study, $^{32}$P-postlabeling was used to detect multiple adducts in mammary fat pads and livers of rats that had been administered 1-NP by gavage. However, DNA adducts resulting from in vitro nitroreduction cochromatographed with *N*-(deoxyguanosin-8-yl)-1-aminopyrene but not with the major adducts found in the mammary fat pads and livers of the treated rats. The investigators

suggested that alternate metabolic pathways, such as ring oxidation or ring oxidation followed by nitroreduction, might be responsible for the formation of these adducts.

As described in Section 10.1.3., rat and dog liver cytosol preparations catalyzed the reduction of 1,3-DNP to several products that were shown to bind to exogenous DNA (Djuric et al., 1985). Furthermore, the addition of acetyl Co-A to the incubations resulted in a 19-fold increase in the binding of metabolites to DNA. The involvement of acetyl Co-A was also demonstrated by increased binding of the 1,3-DNP metabolites, 1-amino-3-nitropyrene, and 1,3-diaminopyrene to tRNA following its addition to rat mammary gland cytosol incubations (King et al., 1986; Imaida et al., 1988).

Djuric et al. (1988) also provided data affirming the correlation between DNA binding of 1-NP or 1,6-DNP and the relative tumorigenicity of the two compounds. Following ip injections of the radiolabeled test compounds, covalent DNA binding was not detected for 1-NP treated animals, but the DNA adduct, *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene was detected in the livers, kidneys, urinary bladder, and mammary glands of rats given 1,6-DNP. Induction of nitroreductases failed to increase the DNA binding by 1,6-DNP, suggesting that additional factors such as O-acetylation may affect the observed DNA binding.

An analysis of DNA binding of 1-NP metabolites following intratracheal instillation in mice was reported by Mitchell (1988). Several adducts were found in the lung, liver, and kidney at 1 day after administration. Upon HPLC analysis, one adduct, accounting for 20% of the total eluted radioactivity in the lung, coeluted with N-(deoxyguanosin-8-yl)-1-aminopyrene (C8-dG-AP). This adduct was also identified in the liver and kidneys. It was also noted that the C8-dG-aminopyrene adduct remained in the lung for as long as 28 days after administration of the 1-NP, suggesting that the persistence of this adduct and/or others may potentially be associated with the induction of lung tumors. These findings suggest that this adduct may result from the nitroreduction of 1-NP in the lung, liver, and/or kidney. These are especially relevant observations when one considers that the lung is a major route of entry for many materials, including diesel exhaust, that contain nitropyrenes.

Based on an increased incidence of mammary tumors in newborn rats, the International Agency for Research on Cancer (1989) considers 4-nitropyrene to be possibly carcinogenic to humans. The genotoxic effects of 4-nitropyrene are summarized in International Agency for

Research on Cancer (1989), but no data were available regarding DNA adduct formation in vivo. However, because of its structural similarity to 1-nitropyrene, it is likely that 4-nitropyrene could be metabolized to intermediates capable of forming DNA adducts.

Although the concentration of DNPs in diesel exhaust is low, some DNPs such as 1,3-DNP, 1,6-DNP, and 1,8-DNP have been shown to be carcinogenic in animals. Furthermore, DNA adduct formation by metabolites of these compounds has been verified. A more complete review of DNP carcinogenicity appears in International Agency for Research on Cancer (1989).

Inhalation studies of 1,3-DNP are limited, but a number of studies have examined its effects following oral and parenteral administration. Subcutaneous administration of 1,3-DNP (>99% purity) to 43 newborn female CD rats (weekly injections over an 8-week period for a total dose of 1.0 mg) resulted in a significant increase (5 of 43 rats, $p < 0.05$) in the incidence of malignant fibrous histiocytomas at the injection site (King, 1988). No such tumors were identified in the dimethylsulfoxide (DMSO) vehicle control group. A similar study reported by Otofuji et al. (1987) used 6-week-old male BALB/c mice to which 1,3-DNP (>99% purity) was administered subcutaneously at dose of 0.05 mg (in DMSO) weekly for 20 weeks. No sc tumors were detected in the treated animals, but the observation period lasted for a maximum of only 60 weeks. Lung, liver, and spleen tumors were identified in the 1,3-DNP-treated mice, and the incidence was significantly greater than that for DMSO controls. In vitro studies have verified DNA binding by 1,3-DNP metabolites, the extent of which could be increased 19-fold if acetyl Co-A was added to the incubation preparations (Djuric et al., 1985).

Both in vivo and in vitro experiments have confirmed DNA adduct formation by metabolites of 1,6-DNP. Twenty-four hours after a single ip dose of [$^3$H]1,6-DNP to preweanling mice, Delclos et al. (1987b) detected the adduct *N*-(deoxyguanosin-8-yl)-1-amino-6-nitropyrene in lung and liver DNA. Male Sprague-Dawley rats receiving an ip dose of [$^3$H]1,6-DNP exhibited DNA adducts in the mammary epithelium, liver, lung, kidney, and urinary bladder. Consistent with these data, Wislocki et al. (1986) reported the development of liver tumors (adenomas and carcinomas) in mice treated with 1,6-DNP. King (1988) reported a significantly increased incidence of malignant fibrous histiocytomas in the peritoneal cavity of rats administered 1,6-DNP intraperitoneally.

Using cytosol preparations from rat or dog liver, Djuric et al. (1985) showed that the resulting 1,6-DNP metabolites (1-amino-6-nitropyrene, 1-nitro-6-nitrosopyrene and 1,6-diaminopyrene) would bind to exogenous DNA. The addition of acetyl Co-A to the incubations produced an additional metabolite, 1-acetylamino-6-nitropyrene, and also greatly increased the extent of its binding to exogenous DNA. The aforementioned metabolites were also detected in rat mammary gland cytosol anaerobic incubations, and their binding to tRNA increased when acetyl Co-A was added to the incubations (King et al., 1986; Imaida et al., 1988).

Results of studies using 1,8-DNP were similar to those for 1,6-DNP. The N-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene adduct was detected in liver and mammary gland cytosol incubations from normal and germ-free CD rats receiving 1.0 $\mu$mol of 1,8-DNP orally (Heflich et al., 1986). The binding was lower in the germ-free rats suggesting the involvement of intestinal microflora in the production of the adduct forming metabolites. Similar to other DNPs, acetyl Co-A increased the extent of metabolite binding to exogenous DNA by rat and dog liver cytosol incubated under anaerobic conditions (Djuric et al., 1985). As with 1,6-DNP, incubation of rat mammary gland cytosol in the presence of acetyl Co-A resulted in metabolites binding to exogenous tRNA (King et al., 1986; Imaida et al., 1988). Several other test systems, including mouse embryo fibroblasts and Chinese hamster ovary cells, also showed DNA adduct formation by 1,8-DNP metabolites (International Agency for Research on Cancer, 1989).

As described in the previous section, 2-nitrofluorene may be metabolized in vivo to products known to be carcinogenic in animal species. However, data are lacking regarding adduct formation by 2-nitrofluorene or its metabolites.

Lung and liver adduct formation in preweanling mice parenterally administered 6-nitrochrysene was reported by Delclos et al. (1987b). The adducts corresponded to the 6-aminochrysene trans-1,2-dihydrodiol adduct produced by microsomal incubations with calf thymus DNA. Incubations using primary cultures of rat liver hepatocytes incubated with 6-nitrochrysene formed two DNA adducts, N-(deoxyguanosin-8-yl)-6-nitrochrysene and N-(deoxyguanosin-8-yl)-6-aminochrysene (Delclos et al., 1987a).

In summary, both in vivo and in vitro studies have demonstrated the formation of adducts involving 1-NP and its metabolites and metabolites of other nitroarenes known to be

constituents of diesel exhaust. Although these data do not provide a definitive description of the mechanism of carcinogenic action, the formation of DNA adducts by known constituents of diesel exhaust affirms that interaction with key cellular components may be possible if these components undergo desorption from the soot particles and become available for biotransformation. The ingestion of particle-associated organics via mucociliary transport following inhalation exposure is demonstrated by their metabolic transformation by intestinal microflora and extrapulmonary tissues and subsequent DNA adduct formation.

## 10.2 PARTICLE EFFECT IN DIESEL EXHAUST-INDUCED CARCINOGENICITY

The role of the carbonaceous core (soot particle) and a particle overload effect in the pulmonary carcinogenesis of diesel exhaust is also of concern. Several studies (Vostal, 1986; Kawabata, 1986; Heinrich, 1990; Wolff et al., 1990; Oberdörster and Yu, 1990) have provided data indicating that the carbonaceous core may have a promotional effect related to the ability of the particle to induce chronic inflammation and promote epithelial cell proliferation. More recent work (Nikula et al., 1991, 1994) has shown that carbon black was also carcinogenic in rats exposed to particle concentrations of 2.5 or 6.5 $mg/m^3$ for 24 mo. The ramifications of particle overload were discussed more fully in the dosimetry chapter (Chapter 4).

A study by Wolff et al. (1990) addressed this topic by comparing the inflammatory responses in rats exposed to diesel exhaust (10 $mg/m^3$) or CB particles (10 $mg/m^3$). Although the level of lung DNA adducts was slightly higher for diesel exhaust exposure, both exposures resulted in inflammatory responses, as determined by increased numbers of neutrophils and macrophages and increased acid proteinase in the bronchoalveolar lavage fluid.

Oberdörster and Yu (1990) evaluated the significance of a particle effect in the tumorigenic response of the lung to diesel exhaust exposure. Using data from studies examining the effects of long-term inhalation exposure to diesel exhaust, $TiO_2$ particles, CB, or toner particles, it was reported that only the surface area of retained particles in the lung showed a reasonable concentration-response relationship relative to tumor incidence and that

particle overload (retained mass or volume of particles) alone may not be the determining factor in lung tumor formation. In this respect, it was shown that particles lacking adsorbed organics (pure CB or $TiO_2$ particles) and diesel exhaust particles exhibited a similar relationship between particle surface area and tumor incidence. The investigators hypothesized a tumorigenic effect would probably require that a "critical" surface area of retained particles be attained for the manifestation of any mechanisms of tumorigenicity.

The possibility of a particle effect in the tumorigenic response has also been demonstrated by Heinrich (1990) in which female Wistar rats (72 per group) were exposed to Printex 90 CB particles for 10 mo followed by a 20-mo exposure-free observation period or for 20 mo followed by a 10-mo exposure-free observation period. A particle concentration of 6.09 $mg/m^3$ was used in both protocols. The Printex 90 particles had an extremely low organic content ($\approx$1,000-fold less than that of diesel exhaust particles). The tumor rates for the 10- and 20-mo exposure durations were 17% (14% malignant) and 8% (all malignant), respectively. Although the lower tumor incidence for the longer exposure period was not consistent, the results demonstrate that the tumor incidences for CB particles with an organic content 1,000-fold less than diesel exhaust particles are equivalent to those reported for diesel exhaust exposures. The fact that these particles were able to exert a significant tumorigenic response implicates the carbon core of diesel exhaust particles as possible tumor initiators in diesel exhaust-induced carcinogenicity at high particle concentrations.

Preliminary findings regarding the importance of the particle-associated organics in the pulmonary carcinogenicity of inhaled diesel exhaust in rats were reported by Mauderly et al. (1991). In this long-term exposure study, rats were exposed 16 h/day, 5 days/week for 24 mo to whole diesel exhaust or CB (free of adsorbed organics) at particle concentrations of 2.5 or 6.5 $mg/m^3$. Controls were exposed to clean air. Lung weights were increased in rats exposed to the highest concentrations of both diesel exhaust or CB but were slightly higher for the diesel exhaust group. The lung burdens of particulate matter were significantly greater for the diesel exhaust-exposed rats at 18 and 23 mo. A substantial transfer of particles from the lungs to lung-associated lymph nodes was observed, but no difference was noted between the diesel exhaust and CB exposure groups. Inflammation and cytotoxicity detected in lavage fluid was greater for diesel exhaust-exposed rats, but the difference was proportional to the higher lung burden of retained particles noted for these animals.

Preliminary data based on approximately 100 male and 100 female rats indicated that the numbers of lung tumors observed grossly at necropsy were nearly identical for the diesel exhaust and CB exposure groups. Tumor type observed included squamous cysts, squamous cell carcinomas, papillary adenocarcinomas, tubular adenocarcinomas, and solid carcinomas. The growth of tumors transplanted into athymic mice has also been similar for diesel exhaust and CB exposures, 74 and 73%, respectively. In summary, these preliminary observations suggest that no difference exists in the type or incidence of lung tumors in rats following long-term exposure to diesel exhaust or CB, and that the particle-associated organics may not significantly involved in the pulmonary carcinogenicity of diesel exhaust in rats.

The carcinogenic potential of many PAHs is well-documented, and, therefore, the potential involvement of PAHs in diesel exhaust-induced carcinogenesis must be considered. However, the recent reports by Heinrich (1990), Mauderly et al. (1991), and Nikula et al. (1994) provide data that call into question the importance of PAHs in diesel exhaust-induced carcinogenesis in these types of experiments that use exceptionally high particle concentrations. Furthermore, a recent report (Bond et al., 1990a; see Section 10.4 for a more detailed discussion of this work) reported that DNA adduct levels were similar in Type II cells of rats exposed either to diesel exhaust or carbon black particles. Although speculative at this time, the information in these studies suggest that PAHs may not be instrumental in diesel exhaust-induced carcingenicity.

## 10.3 POTENTIAL INVOLVEMENT OF PULMONARY LEUKOCYTES IN THE DEVELOPMENT OF LUNG TUMORS

Phagocytic leukocytes have been shown by numerous investigators to be toxic to tumor cells in vivo, and increasing evidence suggests that cells of the mononuclear phagocyte series in particular may be of pivotal importance in providing protection against malignancy in situ. This protective function may, at least in part, result from their ability to produce a tumor necrosis factor (TNF) (Urban et al., 1986). Whether the tumor surveillance and tumoricidal activities of AMs (Hengst et al., 1978; Sone et al., 1983; Sone, 1986; Kan-Mitchell et al., 1985) are compromised or otherwise modified when they are engorged with even relatively benign particles has not been experimentally evaluated. The possibility

remains that diesel and other types of particles at high lung burdens result in decreases in natural killer (NK) cell functional activities in providing defense against tumor formation either by direct particle-cell interactions or by altering the ability of AMs to influence NK cell-mediated host defense against metastatic tumor cells (Sone, 1986). These cells are subpopulations of lymphocytes that possess spontaneous cytolytic activity toward neoplastic cells but not toward normal cells. Moreover, the tumoricidal function of cytotoxic T lymphocytes (Sone, 1986) may be directly or indirectly compromised by the presence of high lung burdens of particles in the lungs.

Phagocytes from a variety of species produce elevated levels of oxidant reactants in response to challenges such as phagocytic stimuli, with the physicochemical characteristics of a phagocytized particle being a major factor in determining the magnitude of the oxidant-producing response. Hatch and co-workers (1980) have demonstrated that interactions of guinea pig AMs with a wide variety of particles including silica, metal oxide-coated fly ash, polymethylmethacrylate beads, chrysotile asbestos, fugitive dusts, polybead carboxylate microspheres, glass and latex beads, uncoated fly ash, and fiberglass increase the production of reactive oxygen species. Similar findings have been reported by numerous investigators for human, rabbit, mouse, and guinea pig AMs (Drath and Karnovsky, 1975; Allen and Loose, 1976; Beall et al., 1977; Lowrie and Aber, 1977; Miles et al., 1977; Rister and Baehner, 1977; Hoidal et al., 1978). As well, polymorphonuclear leukocytes (PMNs) are also known to increase production of superoxide radical, hydrogen peroxide, and hydroxyl radical in response to membrane-reactive agents and particles (Goldstein et al., 1975; Weiss et al., 1978; Root and Metcalf, 1977). Sagai et al. (1993) reported further that diesel exhaust particles were able to produce superoxide and hydroxyl radicals in vitro, without biological activation. Methanol washed particles, in this study, were much less toxic following intratracheal instillation in the mouse, indicating that the active components were extractable with organic solvents. It is well recognized that the deposition of particles in the lung can result in the efflux of PMNs from the vascular compartment into the alveolar space compartment in addition to expanding the AM population size. Following acute exposures, the influx of the PMNs is transient, lasting only a few days (Adamson and Bowden, 1978; Bowden and Adamson, 1978; Lehnert et al., 1988). Strom (1984) has reported that PMNs become abnormally abundant following chronic exposures to particulate diesel exhaust.

In the study by Strom (1984), the numbers of PMNs lavaged from the lungs of diesel-exposed rats generally increased with increasing exposure duration and inhaled exposure mass concentration. Strom (1984) also found that PMNs in diesel-exposed lungs remained persistently elevated for at least 4 mo after cessation of exposure, a potential mechanism for which may be related to an ongoing release of previously phagocytized particles by AMs that engulfed them shortly after deposition. Evidence in support of this possibility has been obtained by Lehnert et al. (1989) in a study in which rats were intratracheally instilled with 0.85, 1.06, or 3.6 mg of polystyrene particles. The PMNs were not found to be abnormally abundant during the clearance of the two lower lung burdens, but they did become progressively elevated in the lungs of the animals in which alveolar-phase clearance was impaired. Moreover, the particle burdens in the PMNs became progressively greater over time. Such findings are consistent with an ongoing particle relapse process, given the relatively short lifespan of PMNs. As previously indicated, lung tumors develop in the rat at lesser lung burdens of diesel exhaust particles than with a particle like $TiO_2$. Polymorphonuclear leukocytes characteristically are increased abnormally in the lung by diesel exhaust exposure, but their presence in the lungs does not appear to be excessive following the pulmonary deposition of even high lung burdens of $TiO_2$ (Strom, 1984; Lee et al., 1986). Thus, the generation of reactive oxygen species by both AMs and PMNs should be considered as one potential factor of what probably is a multistep process that culminates in the development of lung tumors in response to chronic deposition of diesel exhaust particles.

As previously indicated, the production of oxygen species may afford protection against emerging tumor cells by killing the cells, while under other conditions the production of reactive oxygen products conceivably may actually contribute to the development of neoplastic cells. The potential involvement of AMs and PMNs in the development of lung tumors in laboratory rats administered high lung burdens of diesel particles (Mauderly et al., 1987) or having inhaled particles that are generally considered to have low to no cytotoxic potential (e.g., $TiO_2$ [Lee et al., 1986]) over a prolonged period of time may be related to the ability of the lung-free cells to produce reactive oxygen metabolites during phagocytic oxidative metabolism (Hatch et al., 1980). Whereas products of phagocytic oxidative metabolism, including superoxide anion, hydrogen peroxide, and hydroxyl radical can kill

tumor cells (Klebanoff and Clark, 1978), and the reactive oxygen species can peroxidize lipids to produce cytotoxic metabolites such as malonyldialdehyde, some products of oxidative metabolism apparently can also interact with DNA to produce mutations. Along this line, Weitzman and Stossel (1981) found that human peripheral leukocytes were mutagenic in the Ames assay. This mutagenic activity was related to PMNs and blood monocytes; blood lymphocytes alone were not mutagenic. These investigators speculated that the mutagenic activity of the phagocytes was a result of their ability to produce reactive oxygen metabolites, inasmuch as blood leukocytes from a patient with chronic granulomatous diseases, a disease in which neutrophils have a defect in the NADPH oxidase generating system (Klebanoff and Clark, 1978), were less effective in producing mutations than were normal leukocytes. Of related significance in terms of oxygen species being able to cause genetic disturbances, Phillips et al. (1984) demonstrated that the incubation of Chinese hamster ovary cells with xanthine plus xanthine oxidase (a system for enzymatically generating active oxygen species) resulted in genetic damage hallmarked by extensive chromosomal breakage and sister-chromatid exchange and produced an increase in the frequency of thioguanidine-resistant cells (HGPRT test). Aside from interactions of oxygen species with DNA, increasing evidence also points to an important role of phagocyte-derived oxidants and/or oxidant products in the metabolic activation of procarcinogens to their ultimate carcinogenic form (Kensler et al., 1987).

Another characteristic of AMs and PMNs that may contribute to the pathological process is the production of a variety of regulatory and cytotoxic factors. Alveolar macrophages can be stimulated to release several effector molecules or cytokines capable of numerous regulatory functions of other lung cells, including rates of proliferation (Bitterman et al., 1983; Jordana et al., 1988; Denholm and Phan, 1989). The involvement of these factors in diesel exhaust pathology is only beginning to be explored. Release of tumor necrosis factor (TNF) and interleukin-1 (IL-1) are correlated with increasing macrophage particle load (Driscoll and Maurer, 1991). Both share a number of proinflammatory activities, including neutrophil activation and chemotactic effects. Tumor necrosis factor-associated phagocytic cell attraction is mediated through stimulation of lung macrophages; fibroblasts or epithelial cells to secrete the ecosaniods MIP-1*a*, MIP-2, and MCP; peptides that are highly chemotactic to neutrophils; and monocytes (Driscoll et al., 1994). Tumor

necrosis factor has also been implicated in the induction of adhesion molecule expression (Bevilacqua et al., 1989). Finally, TNF also stimulates phagocytic cells to release reactive oxygen species and lysosomal enzymes (Klebanoff et al., 1986). Both $SiO_2$ and $TiO_2$ induced a persistent increase in fibronectin release by lung cells that was consistently correlated with the development of pulmonary fibrosis (Driscoll and Maurer, 1991). Transforming growth factor, TGF-*B*1, present in macrophages and fibroblasts from silica-exposed animals also appears to play a pathogenic role in particle-induced mesenchymal and epithelial lesions (Williams et al., 1993).

A final characteristic of AMs and PMNs that may contribute to the pathologic process leading to lung tumor development following diesel exhaust particle deposition is that these phagocytes are known to release a variety of potentially destructive hydrolytic enzymes, a process known to occur simultaneously with the phagocytosis of particles (Sandusky et al., 1977). The essentially continual release of such enzymes during chronic particle deposition and phagocytosis in the lung may be detrimental to the alveolar epithelium, especially to Type I cells. Evans et al. (1986) showed that injury to Type I cells is followed shortly thereafter by a proliferation of Type II cells. Type II cell hyperplasia is a generally common feature observed in the lungs of animals that have received high lung burdens of various types of particles, including unreactive polystyrene microspheres. Exaggerated proliferation as a repair or defensive response to diesel deposition may have the effect of amplifying the likelihood of neoplastic transformation in the presence of carcinogens beyond that which would normally occur with lower rates of proliferation, assuming an increase in the cell cycling of target cells and the probability of a neoplastic-associated genomic disturbance.

The proliferative response of Type II cells following the deposition of diesel exhaust particles or other types of particles, however, has yet to be directly related to a Type I cell destruction by proteolytic enzymes released by lung phagocytes or to a direct action of particles on the proliferation kinetics of the Type II cells. The production of reactive oxygen species or products therefrom could also be involved in the process. Whatever the stimulus, it remains possible that the lung's AM population may play a role aside from any responsibility for Type I cell damage. Alveolar macrophages have the ability to release several other effector molecules or cytokines that can regulate numerous functions of other lung cells, including their rates of proliferation (Bitterman et al., 1983; Jordana et al., 1988;

Denholm and Phan, 1989). The AM-derived mediators that may stimulate Type II cell hyperplasia following particle deposition in the lung, however, remain to be identified, if in fact the AMs play a regulatory role in the Type II cell proliferative response.

## 10.4 MOLECULAR DOSIMETRY CONSIDERATIONS

An important component in understanding diesel exhaust-induced carcinogenicity is understanding the molecular basis for such an effect. As previously described, considerable data exist regarding DNA adducts resulting from metabolites of organic compounds associated with diesel exhaust particles. For the most part, however, these studies evaluated specific organic compounds or used diesel particulate matter to which had been adsorbed unusually high levels of the organic compounds. This section focuses on those studies evaluating DNA adduct formation in the lungs of animals exposed to diesel exhaust and evaluates dosimetric parameters relating to adduct formation.

DNA adduct formation in the lungs of animals subjected to long-term exposure to whole diesel exhaust has been described by Wong et al. (1986). Using tissues from animals of the Mauderly et al. (1987) study, these investigators reported an increase in DNA adduct formation in male and female F344 rats exposed to whole diesel exhaust (7.1 mg of particles/$m^3$) for 7 h/day, 5 days/week for up to 30 mo. $P_{32}$ postlabeling was applied to DNA that was extracted from six control and six exhaust-exposed rats (males and females). Characterization of the adducts and identification of the exhaust components responsible for their formation were not within the scope of the study. The lungs of exhaust-exposed rats were darkly pigmented and contained diesel-particle-laden macrophages. Aggregates of these macrophages were frequently associated with alveolar wall fibrosis, bronchiolar metaplasia, and, occasionally, squamous metaplasia. Lungs from control rats were not darkly pigmented and had relatively unaltered airway and structure. Autoradiographic analysis revealed elevated levels of DNA adducts in the exhaust-exposed rats. The authors indicated that quantitative and qualitative data regarding DNA adducts resulting from diesel exhaust exposure may be useful for extrapolation to potential effects in humans.

A study by Bond et al. (1989) addressed several key topics regarding the role of DNA adducts in the pulmonary carcinogenicity of diesel exhaust. Using groups of rats exposed to

whole diesel exhaust at particle concentrations of 0, 0.35, 3.5, 7.0, or 10.0 mg/m$^3$ for 12 weeks, the relationship between DNA adduct levels and exposure concentration was examined. The data for the exposure levels employed indicated that DNA adduct formation (about 14 adducts per $10^9$ bases) was similar across all exposure concentrations and was approximately twice that of the sham-exposed group. The fact that DNA adduct formation was independent of exposure concentration may be explained, in part, by previously reported (Bond and Mauderly, 1984) data showing that metabolism of organics associated with diesel exhaust by the isolated perfused rat lung could be saturated at high concentrations, thereby limiting the production of metabolites required for the formation of DNA adducts.

The time course for DNA adduct formation was also examined by Bond et al. (1989). Over a 12-week period of exposure to diesel exhaust (7 mg/m$^3$), lung DNA adducts were found to slowly accumulate. The highest adduct level was reached at 12 weeks, followed by a decline to control level by 4 weeks postexposure. Throughout the exposure period, lung DNA adducts remained constant and at a lower level in sham-exposed rats. The investigators suggested that the rapid repair of adducts relative to their formation might result in a steady-state level of DNA adducts during long-term exposure.

A dosimetry study examined the distribution of DNA adducts in the respiratory tract to determine if increased DNA adduct formation occurred in regions of the lung where diesel exhaust-induced tumors are formed (Bond et al., 1988). For this study, rats were exposed for 12 weeks to diesel exhaust at a particle concentration of 10 mg/m$^3$. The DNA adduct levels were highest in peripheral tissue, which is the same region in which tumors occurred in rats in long-term exposure studies (Mauderly et al., 1987). Although these findings suggest that DNA adduct formation and tumor formation are related, the data do not prove the association.

The previous studies provided data regarding the role of DNA adducts in the pulmonary carcinogenesis of diesel exhaust but were not designed to provide insight into possible target cells. An additional molecular dosimetry study by Bond et al. (1990a) addressed this topic and also compared the effects of diesel exhaust particles with CB particles that were virtually free of the adsorbed organics found on diesel exhaust particles. In this study, rats were exposed to whole diesel exhaust (6.2 mg/m$^3$), CB particles (6.2 mg/m$^3$), or clean air, 16 h/day, 5 days/week for 12 weeks. Relative to clean-air

controls, a significant increase in the total DNA adduct level in Type II cells was noted for rats exposed to diesel exhaust and CB. The exposure to CB and diesel exhaust resulted in an approximate fourfold increase in adduct level compared with controls. However, the investigators noted that there was a large region of unresolved adducts in the chromatograms from diesel-exhaust-exposed rats and that the total adduct level in these animals may be underestimated. Whether the small amount ($\approx$ 0.04%) of extractable organics on the CB particles was responsible for the observed DNA adduct formation or the adducts were the result of inflammatory responses to the particles was not determined. This study does, however, demonstrate that Type II cells are possible targets for diesel exhaust exposure.

Although the actual mechanisms of diesel particle induced cancer induction remain to be elucidated, a general scheme can be hypothesized. First of all, stimulation of chemotaxis and cell adhesion results in aggregation of particle laden macrophages. The oxidants, lysosomal enzymes, eluted organics, and other cytoxic agents released by these macrophages are likely to be concentrated in the immediate region. In fact, alveolar epithelial lesions were reported adjacent to these aggregations in diesel exhaust-exposed rats (Mauderly et al., 1987). In mice, which appear to be less susceptible to the pathological effects of particles, the macrophages remain more disperse (Mauderly, 1994). The role of these various factors in particle-induced cancer induction is still subject to debate. However, it is reasonable to assume that the PAHs will have tumor-initiating effects through direct action on DNA. The cytokines, on the other hand, may cause cell proliferation creating a dividing cell population that may be initiated through exogenous or endogenous process agents. It should be recognized that proliferative effects may include interfering with normal cell death processes.

## 10.5 SUMMARY OF METABOLISM AND MECHANISM OF ACTION OF CARCINOGENIC COMPONENTS OF DIESEL EXHAUST

Currently, a determination of whether the carcinogenicity of diesel exhaust is the result of genetic or epigenetic mechanisms or a combination of both is uncertain (Bond et al., 1989). Based on current data, diesel-exhaust-induced carcinogenesis appears to involve an initiation-promotion mechanism.

The genetic mechanism is supported by data showing the formation of DNA adducts in exposed animals and by the known carcinogenic and mutagenic potential of many of the compounds in diesel exhaust. Several studies affirm the bioavailability from inhaled diesel exhaust particles of compounds such as B[*a*]P and 1-NP, which are known to be carcinogenic or mutagenic. Furthermore, that xenobiotics may undergo biotransformation to reactive intermediates following their entry into the body via inhalation of diesel exhaust particles has been demonstrated for B[*a*]P and various nitroarenes. Results from the metabolism/disposition studies using carbon particles to which organics have been experimentally adsorbed must be interpreted with caution, however. The concentration of organics on these particles is probably much greater than the monomolecular or bimolecular layer on actual diesel exhaust particles and, therefore, might facilitate desorption of the organics from these experimentally prepared particles.

It is generally accepted that one of the underlying mechanisms of carcinogenesis involves the formation of covalent adducts with DNA, resulting in the alteration of cellular genetic information. Several reports have provided data indicating that such adducts are formed in animals following administration of these compounds and after long-term exposure to diesel exhaust. The premise that DNA adduct formation plays a role in diesel exhaust-induced carcinogenesis is substantiated by several findings, including an increase in DNA adducts in the same pulmonary regions where tumors occur, and higher DNA adduct levels in species known to be susceptible to diesel-exhaust-induced tumors. However, the lack of an exposure-response for DNA adduct formation as demonstrated by the molecular dosimetry studies reported by Bond et al. (1990b) suggest the involvement of additional mechanisms. It is clear that an understanding of diesel-exhaust-induced carcinogenicity will require a more complete knowledge of the metabolism and kinetic parameters that relate to adduct formation and the involvement of genetic repair processes.

The presence of a promotional mechanism is supported by the fact that carbon particles per se cause inflammatory responses and increased epithelial cell proliferation and that alveolar macrophage function may be compromised under conditions of particle overload. Studies using CB particles having very low levels of adsorbed organics have been shown to increase inflammatory responses in the lung and to increase adduct formation. Furthermore, recent studies have shown tumor rates resulting from exposures to nearly

organic-free CB particles to be similar to those observed for diesel exhaust exposures, thus providing some evidence for epigenetic mechanisms.

The development of lung tumors in experimental laboratory animals following chronic exposures to diesel exhaust occurs under conditions in which AM-mediated particle clearance from the lung is compromised. As previously noted, tumors have also been found to develop with other types of particles when this clearance mechanism is diminished. Thus, reductions in the functional activity of the lung's alveolar macrophage population in the clearance process generally appear to be intimately related to the carcinogenic response to high lung burdens of particles. Findings that tumors develop in the lungs of laboratory rats at lesser lung mass or volume burdens of diesel particles than with a substance such as $TiO_2$ suggest that the carcinogenic response, however, is not exclusively related to an overabundance of particles in the lungs per se. That the organic components on diesel particles, many of which have demonstrated carcinogenic activity, may be involved in the development of lung tumors is a reasonable hypothesis. The lung's AMs, which phagocytize deposited diesel particles, probably participate in the gradual in situ extraction and metabolism of procarcinogens associated with the diesel particles. Additionally, the normal tumoricidal activities of the AMs may be compromised upon interaction with excessive numbers of diesel particles, and diesel particle-macrophage interactions could lead to the generation of reactive oxygen species that have been shown to be at least mutagenic. Another hypothesis is that the organics are unnecessary for the diesel exhaust-induced tumorigenic effect and that it is the carbon particle alone that is responsible. Both diesel exhaust particles and carbon black particles have greater surface areas and are more effective at tumor induction than are $TiO_2$ particles. Because carbon black particles are essentially devoid of adsorbed organics, the tumorigenic response observed for both of these may be due the effects of the particles alone. The resolution of the relative contributions of chemical carcinogens and particle overload to the tumors occurring in experimental animals exposed to diesel exhaust needs immediate research attention.

Caution must be exercised in extrapolating observations made in animal models to humans. Processes and potential mechanisms discussed herein have largely been derived from animal data. Further research is required to determine how the response of human AMs to particulate diesel exhaust compares with that of AMs in experimental animals at

1 particle concentrations typical of human exposure scenarios. Most important, valid
2 dosimetry for humans requires the elucidation of the underlying mechanisms involved in the
3 development of lung tumors following chronic exposure to whole diesel exhaust.

# REFERENCES


Adamson, I. Y. R.; Bowden, D. H. (1978) Adaptive responses of the pulmonary macrophagic system to carbon: II. morphologic studies. Lab. Invest. 38: 430-438.

Allen, R. C,; Loose, L. D. (1976) Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem. Biophys. Res. Commun. 69: 245-252.

Ball, L. M.; King, L. C. (1985) Metabolism, mutagenicity, and activation of 1-nitropyrene *in vivo* and *in vitro*. Environ. Int. 11: 355-361.

Beall, G. D.; Repine, J. E.; Hoidal, J. R.; Rasp, F. L. (1977) Chemiluminescence by human alveolar macrophages: stimulation with heat-killed bacteria or phorbol myristate acetate. Infect. Immun. 17: 117-120.

Bevilacqua, M. P.; Stengelin, S.; Gimbrone, M. A.; Seed, B. (1989) Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science (Washington, DC) 243: 1160-1165.

Bitterman, P. B.; Adelberg, S.; Crystal, R. G. (1983) Mechanisms of pulmonary fibrosis: spontaneous release of the alveolar macrophage-derived growth factor in the interstitial lung disorders. J. Clin. Invest. 72: 1801-1813.

Bond, J. A.; Mauderly, J. L. (1984) Metabolism and macromolecular covalent binding of [$^{14}$C]-1-nitropyrene in isolate perfused and ventilated rat lungs. Cancer Res. 44: 3924-3929.

Bond, J. A.; Mitchell, C. E.; Li, A. P. (1983) Metabolism and macromolecular covalent binding of benzo[*a*]pyrene in cultured Fischer-344 rat lung type II epithelial cells. Biochem. Pharmacol. 32: 3771-3776.

Bond, J. A.; Butler, M. M.; Medinsky, M. A.; Muggenburg, B. A.; McClellan, R. O. (1984) Dog pulmonary macrophage metabolism of free and particle-associated [$^{14}$C]benzo[a]pyrene. J. Toxicol. Environ. Health 14: 181-189.

Bond, J. A.; Sun, J. D.; Medinsky, M. A.; Jones, R. K.; Yeh, H. C. (1986) Deposition, metabolism, and excretion of 1-[$^{14}$C]nitropyrene and 1-[$^{14}$C]nitropyrene coated on diesel exhaust particles as influenced by exposure concentration. Toxicol. Appl. Pharmacol. 85: 102-117.

Bond, J. A.; Wolff, R. K.; Harkema, J. R.; Mauderly, J. L.; Henderson, R. F.; Griffith, W. C.; McClellan, R. O. (1988) Distribution of DNA adducts in the respiratory tract of rats exposed to diesel exhaust. Toxicol. Appl. Pharmacol. 96: 336-346.

Bond, J. A.; Harkema, J. R.; Henderson, R. F.; Mauderly, J. L.; McClellan, R. O.; Wolff, R. K. (1989) Molecular dosimetry of inhaled diesel exhaust. In: Mohr, U.; Bates, D. V.; Dungworth, D. L.; Lee, P. N.; McClellan, R. O.; Roe, F. J. C., eds. Assessment of inhalation hazards: integration and extrapolation using diverse data: [papers from the 2nd international inhalation symposium]; 1989; Hannover, FRG. Berlin, Federal Republic of Germany: Springer-Verlag; pp. 315-324.

Bond, J. A.; Johnson, N. F.; Snipes, M. B.; Mauderly, J. L. (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, J. A.; Mauderly, J. L.; Wolff, R. K. (1990b) Concentration- and time-dependent formation of DNA adducts in lungs of rats exposed to diesel exhaust. Toxicology 60: 127-135.

Bowden, D. H.; Adamson, I. Y. R. (1978) Adaptive responses of the pulmonary macrophagic system to carbon: I. kinetic studies. Lab. Invest. 38: 422-429.

Boyd, M. R. (1984) Metabolic activation and lung toxicity: a basis for cell-selective pulmonary damage by foreign chemicals. Environ. Health Perspect. 55: 47-51.

Boyland, E. (1980) The history and future of chemical carcinogenesis. Br. Med. Bull. 36: 5-10.

Cantrell, E. T.; Tyrer, H. W.; Peirano, W. B.; Danner, R. M. (1980) Benzo(a)pyrene metabolism in mice exposed to diesel exhaust: II. metabolism and excretion. In: Pepelko, W. E.; Danner, R. M.; Clarke, N. A., eds. Health effects of diesel engine emissions: proceedings of an international symposium, v. 1; December 1979; Cincinnati, OH. Cincinnati, OH: U.S. Environmnetal Protection Agency, Health Effects Research Laboratory; pp. 520-531. EPA report no. EPA-600/9-80-057a. Available from: NTIS, Springfield, VA; PB81-173809.

Chen, K.-C.; Vostal, J. J. (1982) Aryl hydrocarbon hydroxylase induction in rat pulmonary alveolar macrophages (PAM) by diesel particulates or their extracts. Pharmacologist 24: 151.

Conney, A. K. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes memorial lecture. Cancer Res. 42: 4875-4917.

Crapo, J. D.; Young, S. L.; Fram, E. K.; Pinkerton, K. E.; Barry, B. E.; Crapo, R. O. (1983) Morphometric characteristics of cells in the alveolar region of mammalian lungs. Am. Rev. Respir. Dis. 128: S42-S46.

Delclos, K. B.; Miller, D. W.; Lay, J. O., Jr.; Casciano, D. A.; Walker, R. P.; Fu, P. P.; Kadlubar, F. F. (1987a) Identification of C8-modified deoxyinosine and $N^2$- and C8-modified deoxyguanosine as major products of the *in vitro* reaction of N-hydroxy-6-aminochrysene with DNA and the formation of these adducts in isolated rat hepatocytes treated with 6-nitrochrysene and 6-aminochrysene. Carcinogenesis (London) 8: 1703-1709.

Delclos, K. B.; Walker, R. P.; Dooley, K. L.; Fu, P. P.; Kadlubar, F. F. (1987b) Carcinogen-DNA adduct formation in the lungs and livers of preweanling CD-1 male mice following administration of [$^3$H]-6-nitrochrysene, [$^3$H]-6-aminochrysene, and [$^3$H]-1,6-dinitropyrene. Cancer Res. 47: 6272-6277.

Delclos, K. B.; El-Bayoumy, K.; Hecht, S. S.; Walker, R. P.; Kadlubar, F. F. (1988) Metabolic activation of 6-aminochrysene and 6-nitrochrysene: a diol-epoxide of 6-aminochrysene as a probable ultimate carcinogen in preweanling mice. In: King, C. M.; Romano, L. J.; Schuetzle, D., eds. Carcinogenic and mutagenic responses to aromatic amines and nitroarenes: proceedings of the third international conference on carcinogenic and mutagenic *N*-substituted aryl compounds; April 1987; Dearborn, MI. New York, NY: Elsevier; pp. 103-106.

Denholm, E. M.; Phan, S. H. (1989) The effects of bleomycin on alveolar macrophage growth factor secretion. Am. J. Pathol. 134: 355-363.

Djurić, Z.; Fifer, E. K.; Beland, F. A. (1985) Acetyl coenzyme A-dependent binding of carcinogenic and mutagenic dinitropyrenes to DNA. Carcinogenesis (London) 6: 941-944.

Djurić, Z.; Fifer, E. K.; Yamazoe, Y.; Beland, F. A. (1988) DNA binding by 1-nitropyrene and 1,6-dinitropyrene *in vitro* and *in vivo*: effects of nitroreductase induction. Carcinogenesis (London) 9: 357-364.

Drath, D. B.; Karnovsky, M. L. (1975) Superoxide production by phagocytic leukocytes. J. Exp. Med. 141: 257-262.

Driscoll, K. E.; Maurer, J. K. (1991) Cytokine and growth factor release by alveolar macrophages: potential biomarkers of pulmonary toxicity. Toxicol. Pathol. 19: 398-405.

Driscoll, K. E.; Maurer, J. K.; Hassenbien, D.; Carter, J.; Janssen, Y. M. W.; Mossman, B. T.; Osier, M.; Oberdörster, G. (1994) Contribution of macrophage-derived cytokines and cytokine networks to mineral dust-induced lung inflammation. In: Mohr, U.; Dungworth, D. L.; Mauder, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 177-189.

El-Bayoumy, K.; Hecht, S. S. (1983) Identification and mutagenicity of metabolites of 1-nitropyrene formed by rat liver. Cancer Res. 43: 3132-3137.

El-Bayoumy, K.; Hecht, S. S. (1984) Identification of *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene as a major mutagenic metabolite of 6-nitrochrysene. Cancer Res. 44: 3408-3413.

El-Bayoumy, K.; Rivenson, A.; Johnson, B.; DiBello, J.; Little, P.; Hecht, S. S. (1988) Comparative tumorigenicity of 1-nitropyrene, 1-nitrosopyrene, and 1-aminopyrene administered by gavage to Sprague-Dawley rats. Cancer Res. 48: 4256-4260.

Evans, M. J.; Shami, S. G.; Martinez, L. A. (1986) Enhanced proliferation of pulmonary alveolar macrophages after carbon instillation in mice depleted of blood monocytes by Strontium-89. Lab. Invest. 54: 154-159.

Fu, P. P.; von Tungeln, L. S.; Unruh, L. E.; Heflich, R. H. (1986) In vitro metabolism of 4-nitropyrene to a new type of mutagenic metabolite. Pharmacologist 28: 120.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1987) A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 135: 1242-1248.

Glatt, H. R.; Oesch, F. (1976) Phenolic benzo(a)pyrene metabolites are mutagens. Mutat. Res. 36: 379-383.

Goldstein, I. M.; Roos, D.; Kaplan, H. B.; Weissman, G. (1975) Complement and immunoglobulins stimulate superoxide production by human leukocytes independently of phagocytosis. J. Clin. Invest. 56: 1155-1163.

Harris, C. C. (1985) Future directions in the use of DNA adducts as internal dosimeters for monitoring human exposure to environmental mutagens and carcinogens. Environ. Health Perspect. 62: 185-191.

Hatch, G. E.; Gardner, D. E.; Menzel, D. B. (1980) Stimulation of oxidant production in alveolar macrophages by pollutant and latex particles. Environ. Res. 23: 121-136.

Heflich, R. H.; Djuric, Z.; Fifer, E. K.; Cerniglia, C. E.; Beland, F. A. (1986) Metabolism of dinitropyrenes to DNA-binding derivatives *in vitro* and *in vivo*. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 185-197. (Developments in toxicological and environmental science: v. 13).

Heinrich, U. (1990) Results of long-term inhalation exposure of rats to carbon black "Printex 90" [letter to Dr. Lester D. Grant]. Presented at: U.S. Environmental Protection Agency peer review workshop on the Health Assessment Document for Diesel Emissions; July; Research Triangle Park, NC.

Heinrich, U.; Muhle, H.; Takenaka, S.; Ernst, H.; Fuhst, R.; Mohr, U.; Pott, F.; Stöber, W. (1986) Chronic effects on the respiratory tract of hamsters, mice, and rats after long-term inhalation of high concentrations of filtered and unfiltered diesel engine emissions. J. Appl. Toxicol. 6: 383-395.

Hibbs, J. B., Jr.; Chapman, H. A., Jr.; Weinberg, J. B. (1978) The macrophage as an antineoplastic surveillance cell: biological perspectives. J. Reticuloendothel. Soc. 24: 549-570.

Hirose, M.; Lee, M.-S.; Wang, C. Y.; King, C. M. (1984) Induction of rat mammary gland tumors by 1-nitropyrene, a recently recognized environmental mutagen. Cancer Res. 44: 1158-1162.

Hoidal, J. R.; Repine, J. E.; Beall, G. D.; Rasp, F. L., Jr.; White, J. G. (1978) The effect of phorbol myristate acetate on the metabolism and ultrastructure of human alveolar macrophages. Am. J. Pathol. 91: 469-482.

Howard, P. C.; Gerrard, J. A.; Milo, G. E.; Fu, P. P.; Beland, F. A.; Kadlubar, F. F. (1983) Transformation of normal human skin fibroblasts by 1-nitropyrene and 6-nitrobenzo[*a*]pyrene. Carcinogenesis (London) 4: 353-355.

Howard, A. J.; Mitchell, C. E.; Dutcher, J. S.; Henderson, T. R.; McClellan, R. O. (1986) Binding of nitropyrenes and benzo[*a*]pyrene to mouse lung deoxyribonucleic acid after pretreatment with inducing agents. Biochem. Pharmacol. 35: 2129-2134.

Imaida, K.; Tay, L. K.; Lee, M.-S.; Wang, C. Y.; Ito, N.; King, C. M. (1988) Tumor induction by nitropyrenes in the female CD rat. In: King, C. M.; Romano, L. J.; Schuetzle, D., eds. Carcinogenic and mutagenic responses to aromatic amines and nitroarenes: proceedings of the third international conference on carcinogenic and mutagenic *N*-substituted aryl compounds; April 1987; Dearborn, MI. New York, NY: Elsevier; pp. 187-197.

International Agency for Research on Cancer. (1989) Diesel and gasoline engine exhausts and some nitroarenes. Lyon, France: World Health Organization; pp. 189-373. (IARC monographs on the evaluation of carcinogenic risks to humans: v. 46).

Iwai, K.; Udagawa, T.; Yamagishi, M.; Yamada, H. (1986) Long-term inhalation studies of diesel exhaust on F344 SPF rats. Incidence of lung cancer and lymphoma. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 349-360. (Developments in toxicology and environmental science: v. 13).

Jerina, D. M.; Sayer, J. M.; Thakker, D. R.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H. (1980) Carcinogenicity of polycyclic aromatic hydrocarbons: the bay-region theory. In: Pullman, B.; Ts'o, P. O. P.; Gelboin, H., eds. Carcinogenesis: fundamental mechanisms and environmental effects. Dordrecht, The Netherlands: D. Reidel; pp. 1-12. (Jerusalem symposia on quantum chemistry and biochemistry: v. 13).

Jordana, M.; Richards, C.; Irving, L. B.; Gauldie, J. (1988) Spontaneous *in vitro* release of alveolar-macrophage cytokines after the intratracheal instillation of bleomycin in rats: characterization and kinetic studies. Am. Rev. Respir. Dis. 137: 1135-1140.

Kan-Mitchell, J.; Hengst, J. C. D.; Kempf, R. A.; Rothbart, R. K.; Simons, S. M.; Brooker, A. S.; Kortes, V. L.; Mitchell, M. S. (1985) Cytotoxic activity of human pulmonary alveolar macrophages. Cancer Res. 45: 453-458.

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. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 213-222. (Developments in toxicologial and environmental science: v. 13).

Kensler, T. W.; Egner, P. A.; Moore, K. G.; Taffe, B. G.; Twerdok, L. E.; Trush, M. A. (1987) Role of inflammatory cells in the metabolic activation of polycyclic aromatic hydrocarbons in mouse skin. Toxicol. Appl. Pharmacol. 90: 337-346.

King, C. M. (1988) Metabolism and biological effects of nitropyrene and related compounds. Cambridge, MA: Health Effects Institute; research report no. 16.

King, L. C.; Jackson, M.; Ball, L. M.; Lewtas, J. (1983) Binding of 1-nitro[$^{14}$C]pyrene to DNA and protein in cultured lung macrophages and respiratory tissues. Cancer Lett. (Shannon, Irel.) 19: 241-246.

King, C. M.; Tay, L. K.; Lee, M.-S.; Imaida, K.; Wang, C. Y. (1986) Machanism of tumor induction by dinitropyrenes in the female CD rat. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 279-290. (Developments in toxicology and environmental science: v. 13).

King, L. C.; Kohan, M. J.; George, S. E.; Lewtas, J.; Claxton, L. D. (1990) Metabolism of l-nitropyrene by human, rat, and mouse intestinal flora: mutagenicity of isolated metabolites by direct analysis of HPLC fractions with a microsuspension reverse mutation assay. J. Toxicol. Environ. Health 31: 179-192.

Klebanoff, S. J.; Clark, R. A. (1978) The neutrophil: function & clinical disorders. Amsterdam, The Netherlands: Elsevier.

Klebanoff, S. J.; Vadas, M. A.; Harlan, J. M.; Sparks, L. H.; Gamble, J. R.; Agosti, J. M.; Waltersdorph, A. M. (1986) Stimulation of neutrophils by tumor necrosis factor. J. Immunol. 136: 4220-4225.

Lee, K. P.; Henry, N. W., III; Trochimowicz, H. J.; Reinhardt, C. F. (1986) Pulmonary response to impaired lung clearance in rats following excessive $TiO_2$ dust deposition. Environ. Res. 41: 144-167.

Lee, K. P.; Ulrich, C. E.; Geil, R. G.; Trochimowicz, H. J. (1988) Effects of inhaled chromium dioxide dust on rats exposed for two years. Fundam. Appl. Toxicol. 10: 125-145.

Lehnert, B. E.; Valdez, Y. E.; Bomalaski, S. H. (1988) Analyses of particles in the lung free cell, tracheobronchial lymph nodal, and pleural space compartments following their deposition in the lung as related to lung clearance mechanisms. Ann. Occup. Hyg. 32: 125-140.

Lehnert, B. E.; Ortiz, J. B.; London, J. E.; Cline, A. F. (1989) Migratory behavior of alveolar macrophages during clearance of light to heavy lung burdens of particles [abstract]. Am. Rev. Respir. Dis. 139(suppl.): A245.

Leung, H.-W.; Henderson, R. F.; Bond, J. A.; Mauderly, J. L.; McClellan, R. O. (1988) Studies on the ability of rat lung and liver microsomes to facilitate transfer and metabolism of benzo[*a*]pyrene from diesel particles. Toxicology 51: 1-9.

Lowrie, D. B.; Aber, V. R. (1977) Superoxide production by rabbit pulmonary alveolar macrophages. Life Sci. 21: 1575-1583.

Maher, V. M.; Patton, J. D.; McCormick, J. J. (1988) Studies on the metabolism and biological effects of nitropyrene and related nitro-polycyclic aromatic compounds in diploid human fibroblasts. Cambridge, MA: Health Effects Institute; research report no. 17.

Manabe, Y.; Kinouchi, T.; Ohnishi, Y. (1985) Identification and quantification of highly mutagenic nitroacetoxypyrenes and nitrohydroxypyrenes in diesel-exhaust particles. Mutat. Res. 158: 3-18.

Manning, B. W.; Campbell, W. L.; Franklin, W.; Delclos, K. B.; Cerniglia, C. E. (1988) Metabolism of 6-nitrochrysene by intestinal microflora. Appl. Environ. Microbiol. 54: 197-203.

Mauderly, J. L.; Jones, R. K.; Griffith, W. C.; Henderson, R. F.; McClellan, R. O. (1987) Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam. Appl. Toxicol. 9: 208-221.

Mauderly, J. L.; Snipes, M. B.; Barr, E. B.; Bechtold, W. E.; Henderson, R. F.; Mitchell, C. E.; Nikula, K. J.; Thomassen, D. G. (1991) Influence of particle-associated organic compounds on carcinogenicity of diesel exhaust. Presented at: eighth Health Effects Institute annual conference; April; Colorado Springs, CO. Cambridge, MA: Health Effects Institute.

Mauderly, J. L. (1994) Noncancer pulmonary effects of chronic inhalation exposure of animals to solid particles. In: Mohr, U.; Dungworth, D. L.; Mauderly, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 43-55.

McLemore, T. L.; Martin, R. R.; Wray, N. P.; Cantrell, E. T.; Busbee, D. L. (1981) Reassessment of the relationship between aryl hydrocarbon hydroxylase and lung cancer. Cancer (Philadelphia) 48: 1438-1443.

Medinsky, M. A.; Shelton, H.; Bond, J. A.; McClellan, R. O. (1985) Biliary excretion and enterohepatic circulation of 1-nitropyrene metabolites in Fischer-344 rats. Biochem. Pharmacol. 34: 2325-2330.

Medinsky, M. A.; Bond, J. A.; Hunsberger, S.; Sun, J. D. (1988) Lung, liver, and kidney as potential target organs after exposure to 1-nitropyrene, as determined by the time course of covalently bound material. J. Toxicol. Environ. Health 23: 445-454.

Medinsky, M. A.; Bond, J. A.; Hunsberger, S.; Griffith, W. C., Jr. (1989) A physiologically based model of 1-nitropyrene metabolism after inhalation or ingestion. Health Phys. 57 (suppl. 1): 149-155.

Miles, P. R.; Lee, P.; Trush, M. A.; Van Dyke, L. (1977) Chemiluminescence associated with phagocytosis of foreign particles in rabbit alveolar macrophages. Life Sci. 20: 165-170.

Mitchell, C. E. (1982) Distribution and retention of benzo(a)pyrene in rats after inhalation. Toxicol. Lett. 11: 35-42.

Mitchell, C. E. (1988) Formation of DNA adducts in mouse tissues after intratracheal instillation of 1-nitropyrene. Carcinogenesis (London) 9: 857-860.

Möller, L.; Törnquist, S.; Beije, B.; Rafter, J.; Toftgård, R.; Gustafsson, J.-Å. (1987) Metabolism of the carcinogenic air pollutant 2-nitrofluorene in the isolated perfused rat lung and liver. Carcinogenesis (London) 8: 1847-1852.

Möller, L.; Corrie, M.; Midtvedt, T.; Rafter, J.; Gustafsson, J.-Å. (1988) The role of the intestinal microflora in the formation of mutagenic metabolites from the carcinogenic air pollutant 2-nitrofluorene. Carcinogenesis (London) 9: 823-830.

Morrow, P. E. (1986) The setting of particulate exposure levels for chronic inhalation toxicity studies. J. Am. Coll. Toxicol. 5: 533-544.

Morse, M. A.; Carlson, G. P. (1985) Distribution and macromolecular binding of benzo[a]pyrene in SENCAR and BALB/c mice following topical and oral administration. J. Toxicol. Environ. Health 16: 263-276.

Nesnow, S.; Triplett, L. L.; Slaga, T. J. (1984) Tumor initiating activities of 1-nitropyrene and its nitrated products in SENCAR mice. Cancer Lett. (Shannon, Irel.) 23: 1-8.

Nikula, K. J.; Snipes, M. B.; Barr, E. B.; Mauderly, J. L. (1991) Histopathology and lung tumor responses in rats exposed to diesel exhaust or carbon black. In: Annual report of the Inhalation Toxicology Research Institute operated for the United States Department of Energy by the Lovelace Biomedical and Environmental Research Institute: October 1, 1990 though September 30, 1991. Albuquerque, NM: Inhalation Toxicology Research Institute, Lovelace biomedical and Environmental Research Institute; pp. 87-129; report no. LMF-134.

Nikula, K. J.; Snipes, M. B.; Barr, E. B.; Griffith, W. C.; Henderson, R. F.; Mauderly, J. L. (1994) Influence of particle-associated organic compounds on the carcinogenicity of diesel exhaust. In: Mohr, U.; Dungworth, D. L.; Mauderly, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 565-568.

Oberdörster, G.; Yu, C. P. (1991) The carcinogenic potential of inhaled diesel exhaust: a particle effect? J. Aerosol Sci. 21(suppl. 1): S397-S401.

Otofuji, T.; Horikawa, K.; Maeda, T.; Sano, N.; Izumi, K.; Otsuka, H.; Tokiwa, H. (1987) Tumorigenicity test of 1,3- and 1,8-dinitropyrene in BALB/c mice. JNCI J. Natl. Cancer Inst. 79: 185-188.

Perwak, J.; Byrne, M.; Coons, S.; Goyer, M.; Harris, J.; Cruse, P.; DeRosier, R.; Moss, K.; Wendt, S. (1982) An exposure and risk assessment for benzo[a]pyrene and other polycyclic aromatic hydrocarbons. Volume IV. Benzo[a]pyrene, acenaphthylene, benz[a]anthracene, chrysene, dibenz[a,h]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene. Washington, DC: U.S. Environmental Protection Agency, Office of Water Regulations and Standards; EPA report no. EPA-440/4-85-020. Available from: NTIS, Springfield, VA; PB85-222586.

Phillips, B. J.; James, T. E. B.; Anderson, D. (1984) Genetic damage in CHO cells exposed to enzymically generated active oxygen species. Mutat. Res. 126: 265-271.

Pitts, J. N., Jr.; Lokensgard, D. M.; Harger, W.; Fisher, T. S.; Mejia, V.; Schuler, J. J.; Scorziell, G. M.; Katzenstein, Y. A. (1982) Mutagens in diesel exhaust particulate: identification and direct activities of 6-nitrobenzo[*a*]pyrene, 9-nitroanthracene, 1-nitropyrene and 5*H*-phenanthro[4,5-*bcd*]pyran-5-one. Mutat. Res. 103: 241-249.

Plopper, C. G.; Hyde, D. M.; Weir, A. J. (1983) Centriacinar alterations in lungs of cats chronically exposed to diesel exhaust. Lab. Invest. 49: 391-399.

Pott, F.; Stöber, W. (1983) Carcinogenicity of airborne combustion products observed in subcutaneous tissue and lungs of laboratory rodents. Environ. Health Perspect. 47: 293-303.

Randerath, K.; Randerath, E.; Agarwal, H. P.; Gupta, R. C.; Schurdak, M. E.; Reddy, M. V. (1985) Postlabeling methods for carcinogen-DNA adduct analysis. Environ. Health Perspect. 62: 57-65.

Reddy, M. V.; Randerath, K. (1986) Nuclease P1-mediated enhancement of sensitivity of $^{32}$P-postlabeling test for structurally diverse DNA adducts. Carcinogenesis (London) 7: 1543-1551.

Rister, J.; Baehner, R. L. (1977) Effect of hyperoxia on superoxide anion and hydrogen peroxide production of polymorphonuclear leucocytes and alveolar macrophages. Br. J. Haematol. 36: 241-248.

Root, R. K.; Metcalf, J. A. (1977) H2O2 release from human granulocytes during phagocytosis; relationship to superoxide anion formation and cellular catabolism of $H_2O_2$: studies with normal and cytochalasin B-treated cells. J. Clin. Invest. 60: 1266-1279.

Roy, A. K.; El-Bayoumy, K.; Hecht, S. S. (1989) $^{32}$P-postlabeling analysis of 1-nitropyrene-DNA adducts in female Sprague-Dawley rats. Carcinogenesis (London) 10: 195-198.

Saffiotti, U.; Cefis, F.; Kolb, L. H.; Shubik, P. (1965) Experimental studies of the conditions of exposure to carcinogens for lung cancer induction. J. Air Pollut. Control Assoc. 15: 23-25.

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 Radical Biol. Med. 14: 37-47.

Sandusky, C. B.; Cowden, M. W.; Schwartz, S. L. (1977) Effect of particle size on regurgitative exocytosis by rabbit alveolar macrophages. In: Sanders, C. L.; Schneider, R. P.; Dagle, G. E.; Ragan, H. A., eds. Pulmonary macrophage and epithelial cells: proceedings of the sixteenth annual Hanford biology symposium; September 1976; Richland, WA. Washington, DC: Energy Research and Development Administration; pp. 85-105. (ERDA symposium series: no. 43). Available from: NTIS, Springfield, VA; CONF-760927.

Sato, S.; Ohgaki, H.; Takayama, S.; Ochiai, M.; Tahira, T.; Ishizaka, Y.; Nagao, M.; Sugimura, T. (1986) Carcinogenicity of dinitropyrenes in rats and hamsters. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 271-277. (Developments in toxicology and environmental science: v. 13).

Schuetzle, D.; Riley, T. L.; Prater, T. J.; Harvey, T. M.; Hunt, D. F. (1982) Analysis of nitrated polycyclic aromatic hydrocarbons in diesel particulates. Anal. Chem. 54: 265-271.

Sims, P.; Grover, P. L.; Swaisland, A.; Pal, K.; Hewer, A. (1974) Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide. Nature (London) 252: 326-328.

Slaga, T. J.; Viaje, A.; Berry, D. L.; Bracken, W.; Buty, S. G.; Scribner, J. D. (1976) Skin tumor initiating ability of benzo(a)pyrene 4,5- 7,8- and 7,8-diol-9,10-epoxides and 7,8-diol. Cancer Lett. (Shannon, Irel.) 2: 115-121.

Sone, S. (1986) Role of alveolar macrophages in pulmonary neoplasias. Biochim. Biophys. Acta 823: 227-245.

Sone, S.; Brennan, L. M.; Creasia, D. A. (1983) In vivo and in vitro $NO_2$ exposures enhance phagocytic and tumoricidal activities of rat alveolar macrophages. J. Toxicol. Environ. Health 11: 151-163.

Stenbäck, F.; Rowland, J.; Sellakumar, A. (1976) Carcinogenicity of benzo(a)pyrene and dusts in the hamster lung (instilled intratracheally with titanium oxide, aluminum oxide, carbon and ferric oxide). Oncology 33: 29-34.

Stowers, S. J.; Anderson, M. W. (1985) Formation and persistence of benzo(a)pyrene metabolite-DNA adducts. Environ. Health Perspect. 62: 31-39.

Strom, K. A. (1984) Response of pulmonary cellular defenses to the inhalation of high concentrations of diesel exhaust. J. Toxicol. Environ. Health 13: 919-944.

Sun, J. D.; Wolff, R. K.; Kanapilly, G. M. (1982) Deposition, retention, and biological fate of inhaled benzo(*a*)pyrene adsorbed onto ultrafine particles and as a pure aerosol. Toxicol. Appl. Pharmacol. 65: 231-244.

Sun, J. D.; Wolff, R. K.; Kanapilly, G. M.; McClellan, R. O. (1984) Lung retention and metabolic fate of inhaled benzo(*a*)pyrene associated with diesel exhaust particles. Toxicol. Appl. Pharmacol. 73: 48-59.

Sun, J. D.; Bond, J. A.; Dahl, A. R. (1988a) Biological disposition of vehicular airborne emissions: particle-associated organic constituents. In: Watson, A. Y.; Bates, R. R.; Kennedy, D., eds. Air pollution, the automobile, and public health. Washington, DC: National Academy Press; pp. 299-322.

Sun, J. D.; Wolff, R. K.; Maio, S. M.; Barr, E. B. (1988b) The influence of adsorption to carbon black particles on the retention and metabolic activation of benso(a)pyrene in rat lungs following inhalation exposure or intratracheal instillation. Inhalation Toxicol. 1: 79-94.

Tatsumi, K.; Amano, H. (1987) Biotransformation of 1-nitropyrene and 2-nitrofluorene to novel metabolites, the corresponding formylamino compounds, in animal bodies. Biochem. Biophys. Res. Commun. 142: 376-382.

Thyssen, J.; Althoff, J.; Kimmerle, G.; Mohr, U. (1981) Inhalation studies with benzo[*a*]pyrene in Syrian golden hamsters. JNCI J. Natl. Cancer Inst. 66: 575-577.

Tyrer, H. W.; Cantrell, E. T.; Horres, R.; Lee, I. P.; Peirano, W. B.; Danner, R. M. (1981) Benzo(a)pyrene metabolism in mice exposed to diesel exhaust: I. Uptake and distribution. Environ. Int. 5: 307-311.

Urban, J. L.; Shepard, H. M.; Rothstein, J. L.; Sugarman, B. J.; Schreiber, H. (1986) Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. Proc. Natl. Acad. Sci. U.S. A. 83: 5233-5237.

Vostal, J. J. (1986) Factors limiting the evidence for chemical carcinogenicity of diesel emissions in long-term inhalation experiments. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 381-396. (Developments in toxicology and environmental science: v. 13).

Warheit, D. B.; George, G.; Hill, L. H.; Snyderman, R.; Brody, A. R. (1985) Inhaled asbestos activates a complement-dependent chemoattractant for macrophages. Lab. Invest. 52: 505-514.

Weinstein, I. B.; Troll, W. (1977) National Cancer Institute workshop on tumor promotion and cofactors in carcinogenesis. Cancer Res. 37: 3461-3463.

Weiss, S. J.; Rustagei, P. K.; LoBuglio, A. F. (1978) Human granulocyte generation of hydroxyl radical. J. Exp. Med. 147: 316-323.

Weitzman, S. A.; Stossel, T. P. (1981) Mutation caused by human phagocytosis. Science (Washington, DC) 212: 546-547.

White, H. J.; Garg, B. D. (1981) Early pulmonary response of the rat lung to inhalation of high concentration of diesel particles. J. Appl. Toxicol. 1: 104-110.

Williams, A. O.; Flanders, K. C.; Saffiotti, U. (1993) Immunohistochemical localization of transforming growth factor-$\beta$1 in rats with experimental silicosis, alveolar type II hyperplasia, and lung cancer. Am. J. Pathol. 142: 1831-1840.

Wislocki, P. G.; Bagan, E. S.; Lu, A. Y. H.; Dooley, K. L.; Fu, P. P.; Han-Hsu, H.; Beland, F. A.; Kadlubar, F. F. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene, and benzo[a]pyrene in the newborn mouse assay. Carcinogenesis (London) 7: 1317-1322.

Wolff, R. K.; Bond, J. A.; Sun, J. D.; Henderson, R. F.; Harkema, J. R.; Griffith, W. C.; Mauderly, J. L.; McClellan, R. O. (1989) Effects of adsorption of benzo[*a*]pyrene onto carbon black particles on levels of DNA adducts in lungs of rats exposed by inhalation. Toxicol. Appl. Pharmacol. 97: 289-299.

Wolff, R. K.; Bond, J. A.; Henderson, R. F.; Harkema, J. R.; Mauderly, J. L. (1990) Pulmonary inflammation and DNA adducts in rats inhaling diesel exhaust or carbon black. Inhalation Toxicol. 2: 241-254.

Wong, D.; Mitchell, C. E.; Wolff, R. K.; Mauderly, J. L.; Jeffrey, A. M. (1986) Identification of DNA damage as a result of exposure of rats to diesel engine exhaust. Carcinogenesis (London) 7: 1595-1597.

Wood, A. W.; Levin, W.; Lu, A. Y. H.; Yagi, H.; Hernandez, O.; Jerina, D. M.; Conney, A. H. (1976) Metabolism of benzo[*a*]pyrene and benzo[*a*]pyrene derivatives to mutagenic products by highly purified hepatic microsomal enzymes. J. Biol. Chem. 251: 4882-4890.

# 11. QUALITATIVE AND QUANTITATIVE EVALUATION OF THE CARCINOGENICITY OF DIESEL ENGINE EMISSIONS

## 11.1 INTRODUCTION

Concern about the carcinogenic risk of exposure to diesel engine emissions was stimulated in the late 1970s by a report indicating that diesel exhaust is mutagenic (Huisingh et al., 1978), by the knowledge that diesel exhaust contains known carcinogens, and by the projected increase in the use of diesel engines in passenger vehicles. This concern culminated in a U.S. Environmental Protection Agency (EPA)-sponsored quantitative cancer risk estimate for diesel engine emissions (Albert et al., 1983). This study, however, did not include either a qualitative evaluation of carcinogenicity or an evaluation of noncancer health effects of diesel engine emissions. Their estimate, moreover, was based on a "comparative potency" method because of a lack of either chronic animal cancer bioassays or definitive epidemiological data.

Since 1983, several chronic animal inhalation studies and epidemiological investigations designed to assess the carcinogenicity of diesel engine emissions have been completed. These studies are summarized in Chapters 7 and 8. A variety of experiments carried out with the goal of elucidating mechanisms of diesel exhaust-induced carcinogenicity have also been published. Because of the increase in the availability of data and because of the need to provide an up-to-date evaluation of the hazards of diesel exhaust inhalation for the Office of Mobile Sources, a qualitative as well as a quantitative assessment of cancer risk was undertaken. These assessments are presented in this chapter.

The qualitative evidence for carcinogenicity of diesel exhaust is evaluated in Section 11.2. A summary of the International Agency for Research on Cancer (IARC) conclusions is also included (International Agency for Research on Cancer, 1989). This is followed by a review of previous quantitative risk estimates in Section 11.3. A discussion of the exhaust constituents likely to be responsible for cancer induction and their influence on cancer model selection is covered in Section 11.4. The approaches for quantitating risk are outlined in Section 11.5; unit risk estimates are calculated, the results are discussed, and a

unit risk estimate recommended. Both qualitative and quantitative assessments are summarized in Section 11.6.

## 11.2 WEIGHT OF EVIDENCE FOR CARCINOGENICITY OF DIESEL EXHAUST

Lung cancer incidence has been studied in human populations exposed to diesel engine exhaust. An increased incidence of lung cancer was observed in four mortality studies (Howe et al., 1983; Bofetta and Stellman, 1988; Garshick et al., 1988; Wong et al., 1985) and in seven case-control studies (Williams et al., 1977; Hall and Wynder, 1984; Damber and Larson, 1987; Garshick et al., 1987; Benhamou et al., 1988; Hayes et al., 1989; Steenland et al., 1990). A dose response trend was observed in three of the cohort studies (Howe et al., 1983; Bofetta and Stellman, 1988; Wong et al., 1985) and two of the case-control studies (Garshick et al., 1987; Steenland et al., 1990). An increased incidence of lung cancer was not observed in some of the other studies (Waller, 1981; Rushton et al., 1983; Wong et al., 1985; Edling et al., 1987; Lerchen et al., 1987), but each had several methodological limitations such as small sample sizes, short follow-up, lack of adjustment for confounding factors, etc. The studies reporting increased incidences also had some major limitations even though more recent ones, especially those reported by Garshick and co-workers, were able to eliminate most of the confounding variables.

Some epidemiological studies suggest an association between diesel exhaust exposure and lung cancer. Because of the uncertainties created by limited exposure data and the possibility of exposure to other agents, the evidence for carcinogenicity of diesel engine emissions in humans is considered to be limited under Environmental Protection Agency's cancer assessment guidelines (Federal Register, 1986).

In animal experiments, inhalation of whole diesel exhaust resulted in the induction of lung tumors in F344 rats (Brightwell et al., 1986; Ishinishi et al., 1986; Iwai et al., 1986; Mauderly et al., 1987), in Wistar rats (Heinrich et al., 1986b), in Sencar mice (Pepelko and Peirano, 1983), and in NMRI mice (Heinrich et al., 1986b; Stöber, 1986). Lung tumors were also induced by implantation of diesel exhaust condensate in Osborne-Mendel rats (Grimmer et al., 1987). Skin painting of diesel particle extracts induced dermal tumors in

strain "A" mice (Kotin et al., 1955) and in Sencar mice following promotion with tetradecanoylphorbol-13-acetate (TPA) (Nesnow et al., 1982). Extensive studies with *Salmonella* mutagenesis assays have unequivocally demonstrated direct-acting mutagenic activity in both particle extracts and gaseous fractions of diesel exhaust. Positive results have also been reported for gene mutations and chromosome effects in mammalian cell systems after exposure to diesel particle extracts.

Based on the induction of lung tumors via inhalation in at least two strains of rats and two strains of mice and by implantation of diesel exhaust condensate, subcutaneous tumors following injection of exhaust particle organic extracts, and skin tumors following dermal application of exhaust particle organic extracts and supported by positive mutagenicity results, the evidence for carcinogenicity of diesel exhaust in animals is considered to be sufficient under EPA's Cancer Assessment Guidelines (Federal Register, 1986).

The International Agency for Research on Cancer (1989) also evaluated the evidence for carcinogenicity of diesel exhaust and concluded that the evidence for carcinogenicity in humans is limited. This conclusion was based primarily on cohort studies of railroad workers in the United States (Garshick et al., 1988) and Canada (Howe et al., 1983) and two case-control studies (Garshick et al., 1988; Howe et al., 1983) using individuals drawn from the same group of workers. Three further studies of cohorts with less certain exposure to diesel engine exhaust were also considered: two studies of London bus company employees (Rushton et al., 1983; Raffle, 1957) and one of Swedish dock workers (Edling et al., 1987). The IARC concluded that the evidence for carcinogenicity of whole engine exhaust in experimental animals was adequate. Their conclusions were based on positive tumorigenic effects in two different strains of rats in five of six experiments (Karagianes et al., 1981; Iwai et al., 1986; Ishinishi et al., 1986; Heinrich et al., 1986a; Mauderly et al., 1987) and on positive effects in mice in two studies (Pepelko and Peirano, 1983; Stöber, 1986).

The IARC conclusions are thus in general agreement with those of the EPA. Both concluded that human evidence is limited. The IARC considered the animal evidence to be adequate for whole diesel exhaust as well as for extracts of exhaust particles but inadequate for the gaseous phase. Although EPA has not specifically evaluated the gaseous phase and did not consider whole exhaust and particle extracts separately, both agencies agree that whole diesel engine exhaust is carcinogenic in animals.

On the basis of limited evidence for carcinogenicity in humans, diesel engine emissions are considered to best fit into cancer weight-of-evidence Category B1, according to EPA cancer assessment guidelines. This classification is supported by sufficient evidence in animals and positive results in mutagenicity studies and is consistent with the presence of known carcinogens on diesel particles. Chemicals classified in Category B1 are considered to be probable human carcinogens. The International Agency for Research on Cancer (1989) also considers diesel exhaust to be probably carcinogenic in humans.

## 11.3 REVIEW OF PREVIOUS QUANTITATIVE RISK ESTIMATES

Early attempts to assess quantitatively the carcinogenicity of diesel engine emissions were hindered by a lack of both positive epidemiologic studies and long-term animal studies. One means of overcoming these obstacles was the use of the "comparative potency" method (Albert et al., 1983). A second involved estimating risk based on equivocal epidemiological evidence (Harris, 1983).

In the comparative potency method, a combustion product was selected that had a previously determined cancer potency estimate based on epidemiologic data. The ratios of the potency of this agent (e.g., coke oven emissions) to diesel particulate matter extract in a variety of in vivo and in vitro tests are then multiplied by the epidemiology-based potency estimate for coke oven emissions and averaged. If epidemiology-based estimates from more than one pollutant are used, the derived potencies are generally averaged to obtain an overall mean.

The comparative potency estimate of Albert et al. (1983) is probably best known. Their results were obtained using epidemiology-based unit cancer risk estimates for coke oven emissions, cigarette smoke condensate, and roofing tar. Samples of particulate matter were collected from three LDD engines (a Nissan 220 C, an Oldsmobile 350, and a Volkswagen turbocharged Rabbit), all run on a highway fuel economy test cycle, and a heavy-duty engine (Caterpillar 3304) run under steady-state, low-load conditions. The particulate matter was extracted with dichloromethane and tested in a variety of assays. Dose-dependent increases in response were obtained for the four assays listed below:

(1) Ames *Salmonella typhimurium* (TA98) reverse mutation,

(2) gene mutation in L5178Y mouse lymphoma cells,

(3) Sencar mouse skin tumor initiation test, and

(4) viral enhancement of chemical transformation in Syrian hamster embryo cells.

Only the first three assays were used for development of comparative potency estimates because of variability of responses in the enhancement of viral transformation assay. The in vitro studies were carried out both in the presence and absence of metabolic activators. The potency, defined as the slope of the dose-response curve, was measured for each sample in each short-term assay.

The skin tumor initiation test was positive for all the engines tested except the Caterpillar engine. Only the Nissan engine, however, gave strong dose-response data. Because this was considered to be the most biologically relevant test, it was used to derive potency estimates for the Nissan engine. An estimate for the Nissan engine was then derived by multiplying the epidemiology-based potency estimates for each of the three agents (coke oven emissions, roofing tar, and cigarette smoke condensate) by the ratios of their potencies in the skin tumor initiation test to that of the Nissan diesel engine. According to this method, three 95% upper-bound estimates of lifetime cancer risk per micrograms per cubic meter of extractable organic matter were derived for the Nissan diesel, based on potency comparisons with each of the three agents. These values are coke oven emissions, $2.6 \times 10^{-4}$; roofing tar, $5.2 \times 10^{-4}$; and cigarette smoke condensates, $5.4 \times 10^{-4}$. The average of the three is equal to $4.4 \times 10^{-4}$.

The potency of the other diesel emission samples were not estimated directly because of the weak response in the skin tumor initiation test. Instead, their potency relative to the Nissan engine was estimated as the arithmetic mean of their potency relative to the Nissan in the *Salmonella* assay in strain TA98, the sister chromatic exchange (SCE) assay in Chinese hamster ovary (CHO) cells, and the mutation assay in mouse lymphoma cells. The estimated lifetime cancer risk per micrograms per cubic meter for extracts from these engines are as follows: Volkswagen, $1.3 \times 10^{-4}$; Oldsmobile $1.2 \times 10^{-4}$; and Caterpillar, $6.6 \times 10^{-6}$.

To convert these values to a lifetime risk per micrograms per cubic meter of total particulate matter, the results were multiplied by the fraction of extractable organic matter in the particles. This conversion was based on the assumption that the carcinogenic effects were caused solely by the organic fraction. These fractions were Nissan, 0.08; Volkswagen, 0.18; Oldsmobile, 0.17; and Caterpillar, 0.27. After this adjustment, the resulting estimated potencies per micrograms per cubic meter of inhaled diesel particules varied from $3.5 \times 10^{-5}$ for the Nissan to $1.8 \times 10^{-6}$ for the Caterpillar.

Harris (1983) developed comparative potency estimates for the same four engines used by Albert et al. (1983) but used only two epidemiology based potency estimates, those for coke oven emissions and for roofing tar. He used preliminary data from three of the same assays as did Albert et al. (1983), the Sencar mouse skin tumor initiation assay, enhancement of viral transformation in Syrian hamster embryo cells, and the L5178 mouse lymphoma test. The mouse lymphoma test was used both with and without metabolic activation, whereas the Salmonella assay was not used.

The diesel cancer potency estimates by Harris were then derived by multiplying the epidemiology based cancer potency estimates for both coke oven emissions and roofing tar by the ratio of their potencies compared with diesel exhaust particles in each of the four bioassays. For example, the epidemiology-based relative risk of exposure to 1 $\mu g/m^3$ of coke oven emissions was estimated to equal $4.4 \times 10^{-4}/\mu g/m^3$. In the skin tumor initiation test, 2.1 papillomas per mouse were reported for the coke oven sample, compared with 0.53 for the Nissan engine extract. The benzene extractable fraction was assumed to equal 0.06 (slightly less than that in the Albert et al., 1983) studies. The diesel potency estimate using this comparison is then equal to $(0.53/2.1) \times 0.06 \times 4.4 \times 10^{-4}/\mu g/m^3$, or $6.6 \times 10^{-6}/\mu g/m^3$ particulate matter. A total of eight comparisons were made for each engine, four bioassays times two epidemiology-based potency estimates.

The Harris (1983) estimates are not comparable to those of Albert et al. (1983) without adjustment. The unit risk estimates of Albert et al. (1983) are based on absolute risk during lifetime exposure, whereas Harris reported his values in terms of relative risk per year of exposure. To adjust this to lifetime risk for continuous exposure, it is necessary to multiply Harris's values by a factor of $2.7 = 70 \times 0.039$, where 70 reflects the lifetime exposure (70 years), and 0.039 is the lifetime lung cancer mortality rate in the U.S. population.

The range of potencies varied from $0.2 \times 10^{-5}$ to $0.6 \times 10^{-5}$ for the Nissan sample $0.1 \times 10^{-5}$ to $2.4 \times 10^{-5}$ for the Oldsmobile 350, $0.2 \times 10^{-5}$ to $27.8 \times 10^{-5}$ for the Volkswagen Rabbit, and $0.1 \times 10^{-5}$ to $2.5 \times 10^{-5}/\mu g/m^3$ particulate matter for the Caterpillar sample. Harris (1983) derived an overall mean relative risk value of $3.5 \times 10^{-5}/\mu g/m^3$ for the three light-duty engines with a 95% upper confidence limit of $2.52 \times 10^{-4}$. Individual mean values for each engine were not reported. After multiplying by 2.7 to convert to a unit risk, the upper bound estimate of potency of the particulate matter from the three light-duty engines was equal to $6.8 \times 10^{-4}/\mu g/m^3$. McClellan (1986), Cuddihy et al. (1981, 1984), and Cuddihy and McClellan (1983) reported a unit risk of about $7.0 \times 10^{-5}/\mu g/m^3$ using a comparative potency method similar to those reported in the preceding paragraph. The data base was similar to that used by Albert et al. (1983) and Harris (1983). This estimate agrees quite well those reported by Albert et al. (1983). Although the Harris (1983) estimate is somewhat greater, it should be remembered that it was based on preliminary data.

With the availability of chronic cancer bioassays, more recent assessments were based on lung tumor induction in rats. Albert and Chen (1986) reported a risk estimate based upon the chronic rat bioassay conducted by Mauderly et al. (1987). Using a multistage model and assuming equivalent deposition efficiency in humans and rats, they derived a 95% upper confidence limit of $1.6 \times 10^{-5}$ for lifetime risk of exposure to 1 $\mu g/m^3$. Pott and Heinrich (1987) used a linear extrapolation, including data reported by Brightwell et al. (1986), Heinrich et al. (1986b) and Mauderly et al. (1987). They reported risk estimates of $6 \times 10^{-5}$ to $12 \times 10^{-5}/\mu g/m^3$. Most recently, Smith and Stayner (1990), using time-to-tumor models based on the data of Mauderly et al. (1987), derived 95% upper confidence limits ranging from $1.5 \times 10^{-5}$ to $3 \times 10^{-5}/\mu g/m^3$.

At least two attempts were made to estimate lung cancer risk based upon epidemiology data. Harris (1983) also assessed the risk of exposure to diesel engine emissions using data from the London Transport Worker Study reported by Waller (1981). Five groups of employees from the London Transport Authority (LTA) were used. These included bus garage engineers, bus drivers, bus conductors, engineers in central works, motormen, and guards. The first group was considered to have received the highest exposure; the next two, intermediate; and the last two, none. When cancer death rates for the high-exposure group

were compared with those of London males, there was no increase in the observed to expected (O/E) ratios. The author, in fact, considered the results to be negative. Because the low rate of lung cancer in all the LTA exposure groups, however, may have been the result of a "healthy worker" effect, (Harris, 1983) compared the exposed groups with internal controls. He merged the three exposed groups and compared them with the two groups considered to be unexposed. An adjustment was made for the estimated greater exposure levels of garage engineers compared with bus drivers and conductors. Using this method, the relative risk of the exposed groups was greater than 1, but was statistically significant only for garage engineers exposed from 1950 to 1960.

Harris (1983) identified a variety of uncertainties relative to potency assessment based on this study. These included

- small unobserved differences in smoking incidences among groups, which could have a significant effect on lung cancer rates;
- uncertainty about the magnitude of exposure in the exposed groups;
- uncertainty regarding the extent of change in exposure conditions over time;
- random effects arising from the stochastic nature of the cancer incidence; and
- uncertainty in the mathematical specification of the model.

Taking the uncertainties into account, he derived a maximum likelihood relative risk estimate of $1.23 \times 10^{-4}$ with a 95% upper confidence limit of $5 \times 10^{-4}/\mu g/m^3$ particulate matter per year. These estimates are equal to $5 \times 10^{-4}$ and $2 \times 10^{-3}$, respectively, when converted to an absolute risk for lifetime exposure to 1 $\mu g/m^3$ particulate matter. It should be noted that, because of the high degree of uncertainty, the 95% lower confidence limit would predict no risk.

More recently, McClellan et al. (1989) reported risk estimates based on the Garshick et al. (1987) study in which lung cancer in railroad workers was evaluated. Using a proportional risk model, 0.016 excess cancer deaths were estimated to occur for each year of exposure to diesel exhaust. Adjustments were made to convert to continuous exposure (168 versus 40 h) for 70 years. Because exposure levels could not be defined exactly, two

sets of calculations were made, assuming inhaled particle concentrations of either 500 or 125 $\mu g/m^3$. Using an upper 95% confidence limit, the number of excess cancer deaths per year in the United States were estimated to range from 1,900 to 7,400. These values could then be converted to a lifetime risk of exposure to 1 $\mu g/m^3$ diesel exhaust ranging from $0.6 \times 10^{-3}$ to $2 \times 10^{-3}$. Even using the lower 95% confidence limits, an excess of 100 to 400 deaths are predicted, unlike the Harris study in which no excess deaths are predicted. Based upon the lower 95% confidence interval, estimated lifetime risk ranges from $2.7 \times 10^{-4}$ to $10 \times 10^{-4}$. The estimates discussed in this section are listed in Table 11-1.

**TABLE 11-1. ESTIMATED LIFETIME RISK OF CANCER FROM INHALATION OF 1 $\mu g/m^3$ DIESEL PARTICULATE MATTER**

| Method | Potency[a] | Comments | Reference |
|---|---|---|---|
| Comparative potency | $3.5 \times 10^{-5}$ | Nissan engine | Albert et al. (1983) |
| Comparative potency | $2.6 \times 10^{-5}$ | Average of three engines | Albert et al. (1983) |
| Comparative potency | $7.0 \times 10^{-5}$ | Light duty engines | Cuddihy et al. (1984) |
| Comparative potency | $6.8 \times 10^{-4}$ | Average of three engines | Harris (1983) |
| Multistage model | $1.6 \times 10^{-5}$ | Lung cancer rats[b] | Albert and Chen (1986) |
| Straight line extrapolation | $6\text{-}12 \times 10^{-5}$ | Lung cancer rats[c] | Pott and Heinrich (1987) |
| Time-to-tumor model | $2\text{-}3 \times 10^{-5}$ | Lung cancer rats[b] | Smith and Stayner (1990) |
| Logistic regression | $8 \times 10^{-5}$ | Lung cancer rats[d] | McClellan et al. (1989) |
| Epidemiological analysis | $2 \times 10^{-3}$ | London transport study | Harris (1983) |
| Epidemiological analysis | $0.6\text{-}2 \times 10^{-3}$ | Railroad workers | McClellan et al. (1989) |

[a]Estimated upper 95% confidence limit of lifetime risk of continuous exposure to 1 $\mu g/m^3$ diesel exhaust particulate matter.
[b]Used data from studies by Mauderly et al. (1987).
[c]Used data from studies by Brightwell et al. (1986), Heinrich et al. (1986b), and Mauderly et al. (1987).
[d]Used data from studies by Brightwell et al. (1986), Ishinishi et al. (1986), Iwai et al. (1986), and Mauderly et al. (1987).

Each of the three methods used to assess cancer risk have important limitations. In the case of the epidemiology-based estimates, exposure measurements were generally quite limited. It was usually impossible to eliminate all confounding factors. Finally, relative risk

ratios were low, making them more sensitive to the effects of any confounding factors that may be present.

With respect to the comparative potency method, it was assumed that the mix of chemicals responsible for effects of coke oven emissions, cigarette smoke condensate, and roofing tar, have the same relative potency in short-term tests as they do for cancer induction. There is little direct evidence to support this assumption. Of even greater importance is the fact that the comparative potency method is based upon test results from organic extracts of diesel exhaust particulate matter. Recent evidence, as discussed later in the chapter, however, indicates that the inorganic particle core is likely to be primarily responsible for the tumor induction. If this is true, then the biological basis for this method has limited relevance for assessing the cancer potency of inhaled particulate matter.

The animal bioassay-based estimates contain uncertainties inherent in most species extrapolations (i.e., possible differences in metabolism, target organ sensitivity, etc.). Dose extrapolation methods were also relatively crude, and none attempted to estimate concentration at the lung epithelial surface. For example, in only one estimate (Smith and Stayner, 1990) was any adjustment made for species differences in particle deposition efficiency. None of the previous risk estimates accounted for high dose inhibition of particle clearance. Finally, previous estimates were based upon whole diesel exhaust, rather than the fraction most likely to be responsible for cancer induction. In the present exercise, attempts were made to determine which fraction of exhaust is responsible for lung cancer induction and to accurately model the human target organ concentration of this fraction.

## 11.4 APPROACHES TO QUANTITATION OF HUMAN RISK FROM EXPOSURE TO DIESEL EXHAUST

The objective of this section is to assess quantitatively the potential lung cancer risk to humans resulting from exposure to diesel exhaust emissions in ambient air. Ideally, prediction of human risk due to exposure to an environmental pollutant should be made on the basis of human experience. Although several human epidemiological studies on bus, dock, mine, and railroad workers are available, the data from these studies are not adequate for assessing the potential cancer risk to humans from diesel exhaust exposure because of the

lack of reliable information on exposure conditions these workers experienced. Therefore, the challenge to risk assessors is how to provide a "best" risk assessment for diesel exhausts, using all the available information from both animals and humans. In contrast to sparse human data, there is rich information on diesel-induced lung tumors in two strains of rats. An approach to integrate this diverse information is to perform a quantitative risk assessment based on information from animals that includes bioassays and relevant biological mechanisms, and then evaluate the animal-based results against available human experience. This approach is adopted in this report.

In an attempt to quantitatively estimate risk using humans, a detailed analysis of the Garshick et al. (1988) study on railroad workers was carried out. Garshick et al. (1988) analyzed information obtained from the Railroad Retirement Board (RRB) on 55,407 white males who began railroad employment between 1939 and 1949, who were between the ages of 40 and 64 in 1959, and who in 1959 worked at one of the 39 jobs selected to represent a range of potential diesel exhaust (DE) exposure. Two analyses that indicated an effect of exposure to DE on lung cancer risk in this cohort were reported: (1) a relative risk for lung cancer of 1.45 (95% CI = 1.11, 1.89) was observed for DE-exposed workers who were 40 to 44 years of age in 1959 and who consequently had the longest potential exposure to DE (relative risk was progressively lower among DE-exposed workers who were older in 1959 and who had potentially shorter exposures to DE), and (2) the relative risk of lung cancer increased monotonically with increasing duration of work in 1959 or later in a job involving diesel exhaust exposure (disregarding exposures in the current year and in the most recent 4 years); this risk was 1.72 (95% CI = 1.29, 2.23) in the group with the longest exposure (15 to 17 years).

The EPA sponsored an effort to provide a quantitative estimate of lung cancer risk (Crump et al., 1991) on the basis of the epidemiological study by Garshick et al. (1988). Their report is included in this document as Appendix B. More than 50 analyses of the relationship between exposure to diesel exhaust and lung cancer incidence were conducted. However, as detailed in Appendix B, none of these analyses demonstrated a pattern that was consistent with an association between DE exposure and lung cancer; in fact, many of them showed a statistically significant negative association.

It should be pointed out that the failure to find a positive association between the degree of diesel exhaust exposure and lung cancer in the Clement analyses (Crump et al., 1991) does not necessarily negate the positive finding made by Garshick et al. (1988). In fact, the first finding of Garshick et al. (when exposure was classified by "yes" or "no") was supported by these analyses. It is possible that the exposure levels estimated for workers were uncertain enough to obscure a weak but positive association between DE exposure and lung cancer.

Another reason that the data may not be adequate for quantitative risk assessment is that the Crump analyses revealed that there may be an undercount of deaths after 1977. This possibility appears to be supported by a recently released data tape provided by the RRB that includes follow-up of the cohort for additional years, as well as an update of the follow-up through 1980. It is reported that for 1980, about 25% of the deaths on the updated tape were not on the earliest tape (personal communication between Crump and Garshick, 1991). Follow-up of railroad workers' mortality in the Garshick et al. (1988) study was through 1980, which is only 22 years since completion of the conversion of U.S. railroads to diesel. Because the time from first exposure until evidence of an increased risk of environmentally induced lung cancer is often on the order of 20 years, the full impact of DE on lung cancer in this cohort may not be captured by the current study. Therefore, it would be worthwhile to conduct a new study of this cohort to take advantage of the several years of follow-up available. If such a study is conducted, it is recommended that vital status be verified independently of the RRB record.

A second possible approach for developing a quantitative risk assessment would utilize the comparative potency method. This approach was not selected for several reasons. First of all it is not based upon actual measurements of lung cancer. As mentioned previously, it was assumed that the mix of chemicals present in diesel exhaust and the pollutants to which diesel exhaust was compared, have the same potency in short-term tests as they do for cancer induction. The evidence to support this assumption is inadequate. Finally, the short-term measurements used organic extracts of diesel particles rather than particles themselves. As discussed later, the organic fraction, although contributing to, is not likely to be the primary cause of lung cancer.

A third approach and the one selected utilized chronic animal cancer bioassays. This approach was selected because several chronic studies have become available, adequate dose response curves were reported, and the animals were exposed to whole exhaust, unlike the comparative potency tests. Before the derivation of unit risk estimates, however, several issues needed to be addressed. These included (1) determination of the critical target site for diesel exhaust, (2) determination of the fraction of exhaust responsible for tumor induction, (3) selection or development of dosimetric methods for accurately extrapolating dose from experimental animals chronically exposed to high concentrations of exhaust to humans exposed at ambient concentrations, and (4) selection of the most suitable low-dose extrapolation model.

The critical target organ was considered to be the lung. Although Iwai et al. (1986) did report the induction of malignant lymphomas as well as lung tumors in rats following diesel exhaust exposure, the lung was the only target site in other experimental studies with this species. Potential carcinogenic agents present in diesel exhaust may be absorbed from the lungs, enter the bloodstream, and be transported systemically. Data, however, are lacking to evaluate this possibility. Particle adsorbed organics may also reach systemic targets via the GI tract. Particles deposited in the conducting airways are cleared to the mouth quite rapidly and swallowed. A considerable volume of particles are also likely to be ingested as a result of grooming during whole animal exposures (Wolff et. al., 1982) resulting in the possible uptake of carcinogens by the gastrointestinal tract. Because half-times for elution of organics from the particles are considerably longer than passage through the gastrointestinal tract, however, the fraction absorbed is expected to be small. In any case, there is little evidence for systemic effects of diesel exhaust.

The site of action in the lungs is assumed to be the epithelial lining of the alveoli and small airways. According to Mauderly et al. (1987) inflammation and tumors appear to arise from this tissue. Although a connection between interstitial events and lung tumors has been suggested for particles (i.e., fibrosis as a precondition for lung tumors [Kuschner, 1984]), data is unavailable to support this view with respect to diesel exhaust induced tumors.

Accurate extrapolation of dose from experimental studies using animals exposed at high concentrations of exhaust to humans exposed to ambient concentrations requires a variety of adjustments. These include adjustments for species differences in deposition

efficiency and respiratory exchange rates. One of the more important factors and one seldom adjusted for in previous risk estimates is the rate of particle clearance from the deep lung. Normal clearance half-times from the alveolar region are several times longer in humans than rats (Chan et al., 1981; Bohning et al., 1982). This may result in an underestimate of lung burden when extrapolating to humans. On the other hand, the high exposure concentrations used in some of the animal studies resulted in a greatly slowed, or even a complete cessation of clearance (Griffis et al., 1983). In order to accurately extrapolate dose from experimental studies to humans, a detailed dosimetry model developed by Yu et al. (1991) was used. This model is listed in Appendix D. The model accounts for species differences in respiratory exchange rates, deposition efficiency, normal particle clearance rates, transport of particles to lung associated lymph nodes and lung surface area. It also accounts for inhibition of particle clearance due to lung overload. In this model dose is estimated in terms of particle concentration per unit of lung surface area.

Two different approaches were used to derive unit risk estimates. The first one utilized a linearized low-dose extrapolation model (LMS) and is based on the assumption that the insoluble carbon core of the diesel particle is primarily responsible for the carcinogenic effects of diesel exhaust. A particle based assessment was considered to be reasonable for two principal reasons. First of all, exposure to the vapor phase alone did not result in detectable tumor induction in rats (Brightwell et al., 1986; Mauderly et al., 1987; Iwai et al., 1986; Stöber, 1986). Secondly, exposure to carbon black, which is similar in composition to the carbon core of whole diesel exhaust but contains only negligible amounts of organics was about as effective in lung cancer induction as whole diesel exhaust (Heinrich, 1990; Mauderly et al., 1991, Nikula et al., 1994). This issue is considered in more detail in the discussion.

The LMS model is adopted by the EPA as a default procedure to provide an upper bound estimate of risk when data useful to incorporate plausible mechanisms are not available. The model was selected because, although mechanisms of carcinogenesis have been proposed (see discussion section), they remain largely unproven. The Office of Health and Environmental Assessment has developed several stochastic models that can be used to incorporate biological mechanisms if such data are available.

A second approach is based upon the assumption that even though the concentration of carcinogenic compounds on the diesel particles is small, they nevertheless can act in concert with the particles to induce carcinogenesis. An alternative low-dose extrapolation model was developed, which allows for the possibility that organic materials may induce organ specific adducts which contribute to the carcinogenic process. This model was developed because it was recognized that various PAHs and nitroaromatics are present, even if at low concentrations, and may contribute to cell initiation. This model is outlined in detail in Appendix C.

Quantitative assessments are presented in Section 11.5. Both approaches selected utilize the detailed dosimetry model previously mentioned to estimate concentration of particulate matter at airway and alveolar surfaces. Risk is based upon the assumption that an equivalent concentration of particles per unit of lung surface area results in equivalent risk in humans and rats. In one low dose extrapolation model, the linearized multistage, dose is based on the carbon particle minus adsorbed organics and on the carbon particle with the organics present in an alternative model.

## 11.5 DOSE-RESPONSE CALCULATIONS BASED ON ANIMAL BIOASSAY DATA

Calculation of unit risk estimates, is provided in this section. Unit risk is a quantification of the carcinogenic potency for the compound. The unit risk estimate for an air pollutant is defined as the 95% upper bound of the increased lifetime cancer risk for an individual continuously exposed for a lifetime to an air pollutant at a concentration of 1 $\mu g/m^3$ in ambient air. The results of the unit risk calculations are summarized in Table 11-6.

### 11.5.1 Data Available for Risk Calculations

As reviewed in Chapter 7, five bioassay studies showed positive lung tumor responses in rats (Brightwell et al., 1986; Ishinishi et al., 1986; Iwai et al., 1986; Stöber, 1986; Mauderly et al., 1987). Only three of them (Tables 11-2 through 11-4) were used for unit

**TABLE 11-2. INCIDENCE OF LUNG TUMORS IN FISCHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO HEAVY-DUTY ENGINE EXHAUST**

| | Dose Metric | | |
|---|---|---|---|
| Exposure Concentration ($mg/m^3$) | Weekly Exposure ($mg/m^3 \times h$)[a] | Lung Particle Burden ($mg/cm^2$ lung surface)[b] | Lung Tumor Incidence |
| 0 | 0 | 0 | 2/230 |
| 0.35 | 12 | $6.4 \times 10^{-5}$ | 3/223 |
| 3.50 | 122 | $2.8 \times 10^{-3}$ | 8/222 |
| 7.08 | 248 | $6.0 \times 10^{-3}$ | 29/227 |

[a]Exposures were 7 h/day, 5 days/week.
[b]Calculated using mathematical models in Appendix D.

Source: Mauderly et al. (1987).

**TABLE 11-3. INCIDENCE OF LUNG TUMORS IN FISCHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO HEAVY-DUTY ENGINE EXHAUST**

| | Dose Metric | | |
|---|---|---|---|
| Exposure Concentration ($mg/m^3$) | Weekly Exposure ($mg/m^3 \times h$)[a] | Lung Particle Burden ($mg/cm^2$ lung surface)[b] | Lung Tumor Incidence |
| 0 | 0 | 0 | 1/123 |
| 0.46 | 44 | $2.5 \times 10^{-4}$ | 1/123 |
| 0.96 | 92 | $2.0 \times 10^{-3}$ | 0/125 |
| 1.84 | 177 | $4.2 \times 10^{-3}$ | 4/123 |
| 3.72 | 357 | $8.8 \times 10^{-3}$ | 8/124 |

[a]Exposures were 16 h/day, 6 days/week.
[b]Calculated using mathematical models in Appendix D.

Source: Ishinishi et al. (1986).

**TABLE 11-4. INCIDENCE OF LUNG TUMORS IN FISCHER 344 RATS (MALES AND FEMALES COMBINED) EXPOSED TO DIESEL ENGINE EXHAUST**

| | Dose Metric | | |
|---|---|---|---|
| Exposure Concentration ($mg/m^3$) | Weekly Exposure ($mg/m^3 \times h$)[a] | Lung Particle Burden ($mg/cm^2$ lung surface)[b] | Lung Tumor Incidence |
| 0 | 0 | 0 | 4/250 |
| 0.7 | 56 | $3.5 \times 10^{-4}$ | 1/112 |
| 2.2 | 176 | $4.2 \times 10^{-3}$ | 14/112 |
| 6.6 | 528 | $1.3 \times 10^{-2}$ | 55/111 |

[a]Exposures were of 16 h/day, 5 days/week.
[b]Calculated using mathematical models listed in Appendix D.
[c]The number of animals sacrificed at 6 and 12 mo are excluded from the denominators.

Source: Brightwell et al. (1986).

risk calculations because each study selected was designed using multiple exposure groups studies of animals exposed to carbon black, although not used in the risk calculations, did influence the methodology. The time-to-event (i.e., death with or without tumors) data are available for the Mauderly et al. (1987) study. These time-to-event data are used in all the risk calculations based on the Mauderly et al. (1987) data.

## 11.5.2 Calculation of Unit Risks

The linearized multistage (LMS) model used in approach number one to calculate particle based risk estimates has the mathematical form $P = 1 - \exp(-Z)$, where Z is either $Z = q_0 + q_1xd + \ldots + q_mxd^m$, a polynomial of concentration d; or $Z = (Q_0 + Q_1xd + \ldots + Q_mxd^m)\ xt^k$, a polynomial of concentration d multiplied by a time factor $t^k$ and thus is more appropriate for risk calculations. The availability of preliminary data from when time-to-event data are used. When time-to-event data are used, the lifetime risk is calculated by actuarial life table approach using the survival probability of the NTP control animals (Fischer 344 rats) provided in Portier et al. (1986). The range of extrapolation is about three orders of magnitude in the present study. Denote $P_0$ the lifetime cancer risk at

concentration 0. Because the extra risk $(P - P_0)/(1 - P_0)$ is dominated by the linear term $q_1xd$ at low concentration, the 95% upper bound of $q_1$ is used to represent unit risk when d is expressed in micrograms per cubic meter.

When extrapolating risk from animals to humans, a dose metric that will induce the same tumor incidence rate in animals and humans must be assumed (i.e., dose equivalence assumption). The method used for calculating equivalent doses uses a mathematical model to adjust for the dosimetric parameters determining lung burden of particulate matter in rats and humans and to correct to a dose per unit lung surface area. In this method, the dose per unit lung surface area is considered to be equally potent in the induction of lung tumor responses in both animals and humans. The deposition-clearance-retention model used to estimate dose per unit lung surface area is described in Appendix D. This model accounts for animal-human differences in regional deposition efficiency; respiratory exchange rates; particle clearance rates at low doses, as well as at doses resulting in impaired clearance; and lung surface area. In these calculations, the mass fraction of the total particle adsorbed organics is assumed to be 20%, with half the mass composed of slowly eluted organics ($t_{1/2}$ = 30 days) and the other half composed of rapidly eluted organics ($t_{1/2}$ = 1.3 h). The remainder is considered to be inorganic carbon.

The effect of high exposure concentrations with an accompanying impairment of clearance on lung burden of organics is shown in Figure 11-1 and on lung burden of particulate matter in Figure 11-2. The data are plotted in terms of estimated lung burden per unit of exposure. Little or no overload is seen at the lowest exposure concentration. As a result the modeled lung burdens of both organics and carbon particulate matter reach an asymptote after a few weeks of exposure. At higher concentrations particle clearance slows and may even stop. In this case, lung particles burdens increase continually during exposure. The organic constituents, on the other hand, elute fairly quickly from the particles and reach a steady state even during continued exposure at high concentrations. Lung burdens of organics are therefore less affected by overload inhibition of clearance.

Most of the tumor incidence data in the animal experiments were recorded at exposure concentrations resulting in some degree of particle clearance inhibition. Unless adjustment for clearance rates are made, lung particle burdens during low-dose extrapolation will be

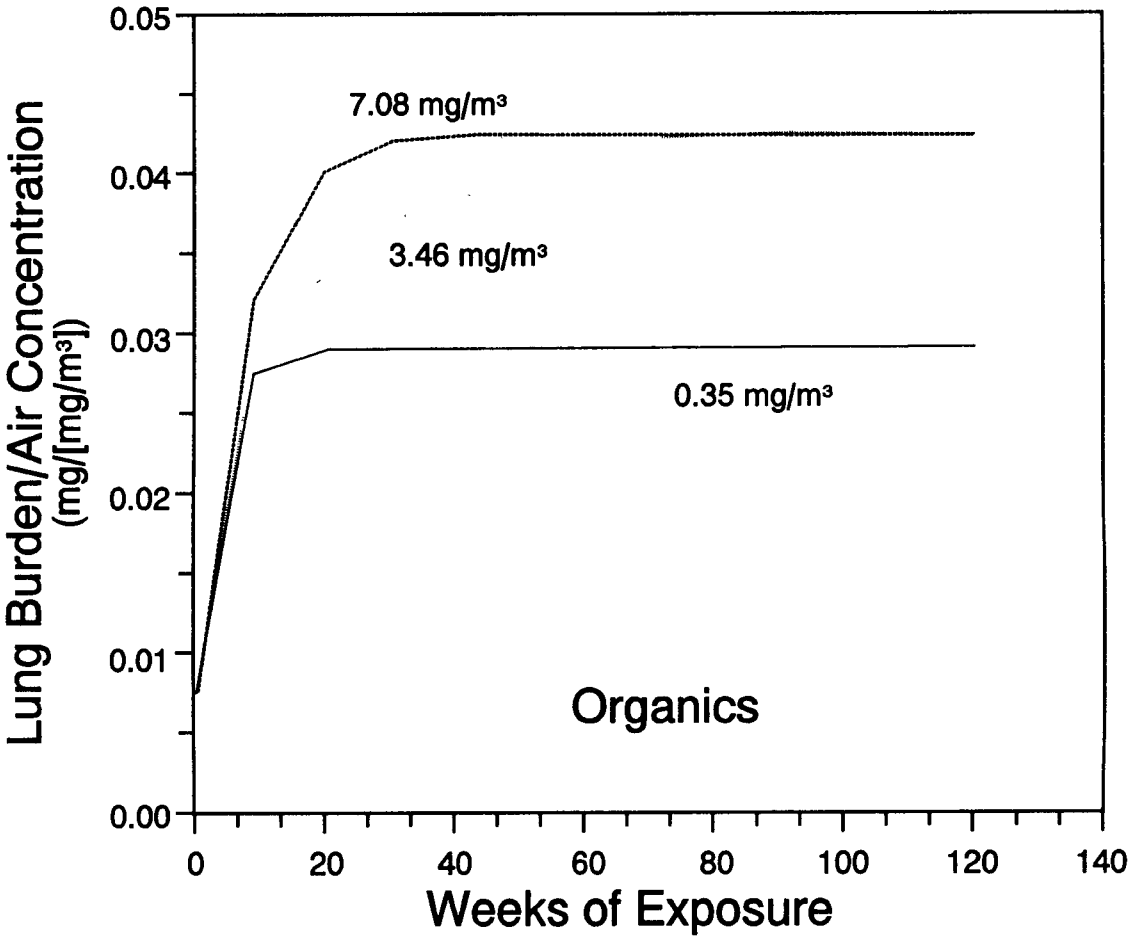


**Figure 11-1. Calculated lung burden (organic matter) in rats exposed (7 h/day, 5 days/week) to three different concentrations of particulate matter.**

overestimated. On the other hand, because lung burdens of the organic fraction are less influenced by clearance rates, they will decline at a differing rate from particles during low-dose extrapolation. The fraction of exhaust selected for development of cancer potency estimates can therefore affect results.

Determination of dose for the carbon core becomes problematic because the lung burdens over time differ drastically between low- and high-exposure concentrations. As can be seen in Figure 11-2, a steady state is reached only for the low-exposure concentrations. The difference in the behavior of lung burden over time between low- and high-exposure groups suggests that the use of a lung burden at a fixed time point (e.g., at 1 year after exposure began) as an estimate of dose may not be appropriate. In this report, the average lung burden (in milligrams) is used as the target organ dose. This is calculated by dividing

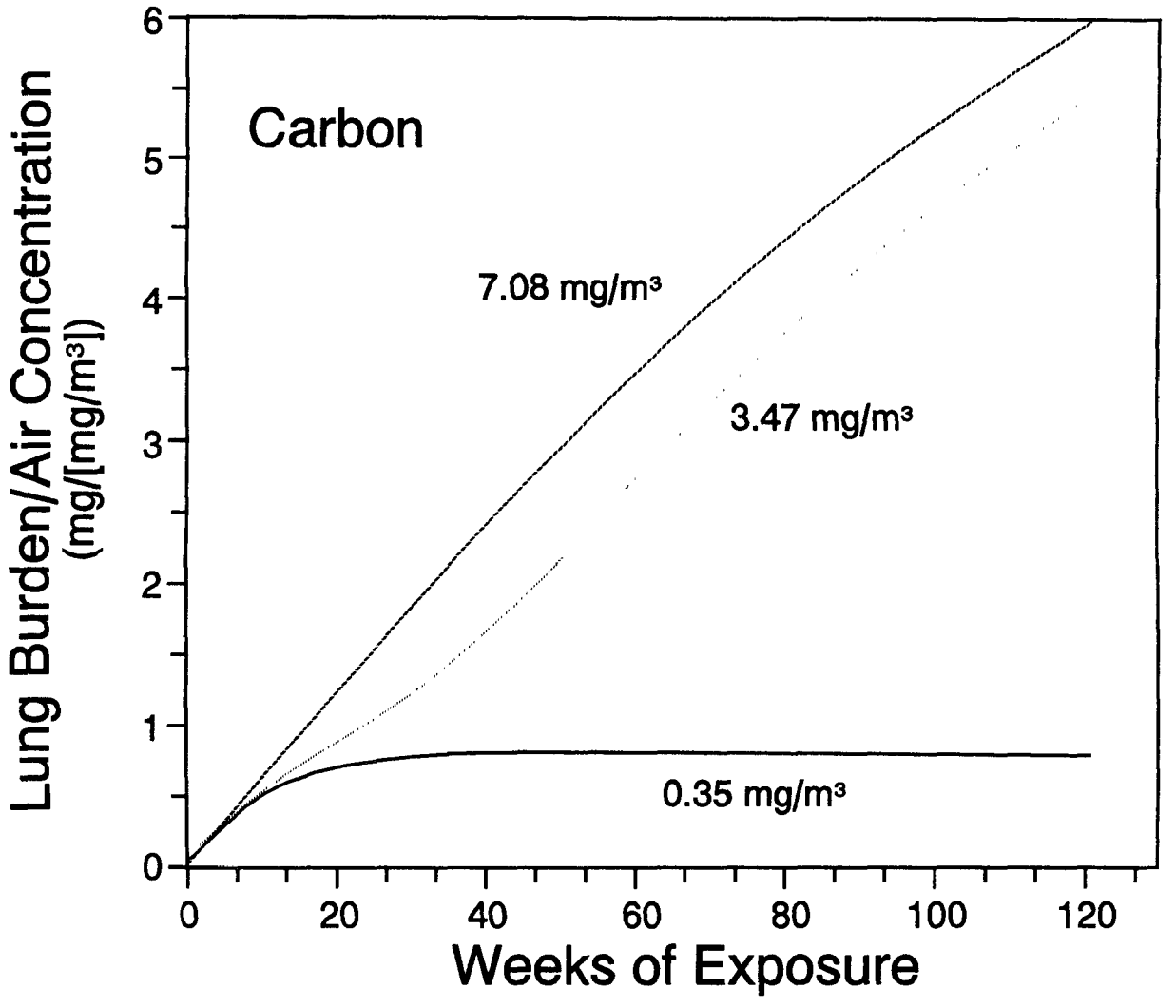


**Figure 11-2. Calculated lung burden (carbon core) in rats exposed (7 h/day, 5 days/week) to three different concentrations of particulate matter.**

the area under the curve (of lung burden over time) by the corresponding time period (156 weeks) over which area under the curve is calculated.

A second approach to estimating risk was attempted because it was considered more desirable to base risk upon a biologically based dose-response model. Although the data are presently insufficient to replace the linearized multistage model, the implications of hypothetical mechanisms of cancer induction proposed at a 1992 EPA workshop on particles can nevertheless be investigated. The biological issues considered included the role of particle adsorbed organics as well as that of a variety of mediators secreted by particle laden

macrophages upon the carcinogenic process. A stochastic model was therefore developed with the following properties:

(1) It accounts for the possible effects of both the carbon particles and its associated organics.

(2) It allows evaluation of the contribution of both carbon particles and organics to tumor induction.

(3) It allows for changing parameters with increasing lung burden.

(4) It was assumed that cell proliferation and tumor induction are stochastic. For instance, it is not appropriate to assume that all cells divide at the same rate.

This model, which is illustrated in detail in Appendix C, allows for initiating properties of both the carbon and the organic fraction and for the proliferative effects of the carbon fraction. Although the mechanisms remain to be proven, it is assumed that carcinogenic agents present in the organic fraction act directly upon the target cells, primarily via initiation. It is further assumed that the majority of the particles are ingested by macrophages. Particle laden macrophages are then induced to secrete a variety of mediators (i.e., reactive oxygen species, cytokines, etc.), which diffuse to the target cells inducing initiation, proliferation, and conversion of initiated cells to malignant cells.

There is considerable uncertainty regarding particle effects at low doses. It has been claimed that particle-induced initiation and/or proliferation does not occur at low doses, (i.e., secretion of mediators only occurs when macrophages are overloaded with particles) (Vostal, 1986). There is inadequate evidence, at present, to support or refute this claim. Moreover, even if macrophage overload is required, because of uneven distribution of particles, some macrophages may become overloaded even at low exposure concentrations. Because of this uncertainty the alternative model, like the LMS model, does not depart from linearity at low doses.

The tumorigenic results reported by Mauderly et al. (1987) were the only data used to estimate model parameters. Mauderly's data are the most useful because they contain information on natural mortality and serial sacrifice of animals, a valuable tool for estimating tumor latency. The model parameters are based upon the development of malignant tumors,

rather than all tumors as was done in the first method. This was necessary in order to utilize mortality data. Lung burdens were calculated with the aid of the same dosimetry model used for the linearized multistage (LMS) derived estimates.

### 11.5.3 Results of Unit Risk Calculations

Unit risks, based upon the LMS approach, were calculated using tumor incidence data from each of the three bioassays (Brightwell et al., 1986; Ishinishi et al., 1986; and time-to-event data from Mauderly et al., 1987) and the corresponding equivalent doses (Tables 11-2 through 11-4). The resultant unit risk estimates are listed in Table 11-5. They range from $1.6 \times 10^{-5}$ to $7.1 \times 10^{-5}/\mu g/m^3$ with a geometric mean of $3.4 \times 10^{-5}/\mu g/m^3$. The unit risk is defined as the 95% upper confidence limit of the risk of lung cancer mortality from lifetime exposure to 1 $\mu g/m^3$ of diesel exhaust particulate matter.

**TABLE 11-5. UNIT RISK ESTIMATES PER MICROGRAMS PER CUBIC METER OF DIESEL EXHAUST**

| | 95% Upper Confidence Limits of the Cancer Risk from Exposure to 1 $\mu g/m^3$ Diesel Particulate Matter |
|---|---|
| Mauderly et al. (1987) | $3.4 \times 10^{-5}$ |
| Ishinishi et al. (1986) | $1.6 \times 10^{-5}$ |
| Brightwell et al. (1986) | $7.1 \times 10^{-5}$ |
| Geometric mean of three studies | $3.4 \times 10^{-5}$ |

[a]If milligrams per lung weight, instead of milligrams per lung surface is used as equivalent dose, those risk estimates are reduced by a factor of 4.

In these calculations, the relationship between air concentration (in micrograms per cubic meter) and lung burden (in milligrams) in humans is used to determine the human lung burden resulting from inhalation of 1 $\mu g/m^3$ of diesel exhaust particulate matter. The particle burden in terms of mass/unit lung surface area is then multiplied by the slope factor derived from the animal data. For instance, when rat data from Ishinishi et al. (Table 11-3) are used, the carcinogenic slope for rats (i.e., the 95% upper confidence limit of the linear coefficient in the multistage model) expressed in terms of equivalent dose (micrograms of

carbon particulate matter per square centimeter) is $8.3 \times 10^{-3}/\mu g/cm^2$. According to the dosimetry model, an air concentration of 1 $\mu g/m^3$ of particulate matter corresponds to a mass of 1,230 $\mu g$ of carbon particles per human lung. Because the lung epithelial surface area, including the alveolar region and conducting airways, is assumed to equal 627,000 $cm^2$, the unit risk of $1.6 \times 10^{-5}/\mu g/m^3$ is derived by multiplying $8.3 \times 10^{-3}/\mu g/cm^2 \times$ 1,230 $\mu g/627{,}000\ cm^2$.

The unit risk estimate derived using the alternate model and based upon the malignant tumor data from the Mauderly et al. (1987) study is equal to $1.65 \times 10^{-5}/\mu g/m^3$. This is lower than the estimate of $3.4 \times 10^{-5}/\mu g/m^3$ derived from the same study, based on the LMS approach (Table 11-5). Application of the LMS model using only malignant tumors from the Mauderly et al. study, rather than all lung tumors, however, resulted in a unit risk estimate of $1.74 \times 10^{-5}/\mu g/m^3$. Thus, the unit risk estimates for the two approaches are identical ($1.7 \times 10^{-5}/\mu g/m^3$ when rounded to two significant figures). In fact, 1.7 cannot be considered to differ from $3.4 \times 10^{-3}/\mu g/m^3$ because of the range of uncertainty. The estimated risks may differ somewhat with increasing doses, since the slopes are not identical at all exposure levels. See Appendix C for details.

## 11.5.4 Discussion of Unit Risk Estimates

### 11.5.4.1 Basis for the Present Approach

In most of the earlier evaluations, species differences in dosimetric parameters, such as deposition efficiency and particle clearance rates were not accounted for. Of particular importance, failure to account for overload inhibition of clearance as well as species difference in lung particle clearance can result in erroneous estimates of low-dose lung burdens in humans and thereby risk. Despite the greater uncertainty in the earlier estimates, the ones based upon chronic animal bioassays do not differ greatly from the present one (Table 11-1). They also fall within the same range as those derived using the comparative potency method.

The human-epidemiology-based cancer risk estimates were, in most cases, greater than animal-based ones. Exposure measurements, however, were quite limited in most of these studies. Moreover, because of the very small increases in relative risk ratios, any degree of confounding for smoking or other reasons can result in considerable error in the unit risk

estimates. Finally, as discussed previously, after an exhaustive evaluation of the Garshick et al. (1987, 1988) epidemiology data, the analysis included in Appendix B was unable to obtain positive dose response curves. The uncertainty in the previously published risk estimates is therefore considered to be quite high.

The risk estimates using both the LMS model as well as the alternative model are based on the dose equivalence assumption that the same lung surface concentration (in milligrams per square centimeter) of particulate matter will lead to the same effect with respect to lung tumor induction in both the rat and human lung epithelial cells. This is considered to be a more accurate estimate of target organ dose then the one derived using the older EPA approach. The use of surface area also was considered to be superior to lung weight because lung tumors arise from epithelial cells rather than from interstitial cells.

If diesel exhaust induced lung tumors in humans originate in conducting airways rather than alveoli, it could be argued that a dose based on surface area of conducting airways, rather than the lower respiratory tract (LRT) surface, is more appropriate. Because it is believed that tumor induction is related in some way to the particle-laden macrophages residing in alveolar regions, it is assumed that exhaust-induced lung tumors in humans are more likely to arise from the alveolar region. Thus, surface area of the LRT, which to a large extent consists of alveolar surface, is considered to be the most appropriate dosimetric parameter.

The risk estimates are also based upon the assumption that sensitivity of target organ cells among species is equal. This is a departure from past EPA policy to adjust dose according to body weight to the two-thirds power. Adjustment was based on the assumption that slower rates of metabolism with accompanying slower rates of detoxification and/or repair mechanisms renders the human cell more sensitive than cells from a smaller mammal. It is unlikely in the current situation, however, that the primary cause of cancer is via direct-acting carcinogens. More likely, epithelial cell transformation is induced by various factors secreted by the particle-laden macrophages, possibly in conjunction with partially activated organic agents. In this case, the greater sensitivity of human lung epithelial cells may be offset as a result of slower production of these transforming agents and/or chemical activation by the human alveolar macrophage. Obviously, these assumptions have not been proven and are subject to challenge. The actual agent or agents responsible for the

diesel-induced lung tumor induction and the mechanisms of action have still not been determined with any degree of certainty. Nevertheless, the evidence to date does not support a further correction based on metabolic rate. Results of future research may establish an appropriate factor, which could then be included.

Neither approach accounts for the possibility of cancer induction by the vapor phase chemicals. There is little evidence to support the likelihood that this component contributes significantly to the tumorigenic effects. Although benzene (Schuetzle and Frazier, 1986) as well as aldehydes including acetaldehyde, acrolein, benzaldehyde, and formaldehyde (Smith, 1989) are contained in this fraction, the concentration of these known or suspected carcinogens are quite low and unlikely to induce detectable tumorigenic responses at the exhaust dilutions used. The sites of tumor induction by these chemicals also differ from those induced by diesel exhaust. Benzene induces leukemia in humans (Rinsky et al., 1981), zymbal gland tumors in rats and mice, lymphomas in mice and oral tumors in rats (Huff et al., 1989). Aldehydes generally induce nasal tumors (Swenberg et al., 1980). Lung tumors, however, have not been reported in any of these studies. Finally, exposure to exhaust filtered to remove particles generally did not result in a detectable increase in tumor incidence in rats (Brightwell et al., 1986; Mauderly et al., 1987).

Although halogenated dioxins (PCDDs) and dibenzofurans (PCDFs) are emitted in many combustion processes, output from diesel engine are uncertain. Marklund et al. (1990) were unable to measure any PCDDs or PCDFs in diesel engine emissions. Their detection limits, however, were quite high due to technical difficulties. Jones (1993) cited data from the California Air Resources Board, in which a diesel bus and a heavy duty truck were reported to emit 1.6 and 4.9 ng/km, respectively. Oehme et al. (1991) estimated that heavy duty diesel powered vehicles emitted between 0.8 and 9.5 ng/km PCDD/PCDF based upon measured air concentrations along with differential counts of diesel and gasoline powered vehicles in a highway tunnel. Results of such an indirect method, however, must be considered to be highly uncertain.

There is even less data on ambient levels of PCDDs and PCDFs due to engine emissions. Concentrations of PCDD/PCDFs were reported to be less than one picogram per cubic meter at the tunnel exit in the Oehme et al. (1991) study. Because concentrations would be expected to be considerably lower along open roads, these agents are unlikely to be

present in sufficient quantities to contribute significantly to adverse health effects from diesel exhaust.

Although the vapor phase components failed to induce lung cancer in rats, the possibility of interactive effects remains. Heinrich et al. (1982), for example, reported data suggesting that the gaseous fraction of diesel exhaust promoted the tumorigenic effects of dimethylnitrosamine in Syrian hamsters. Unfortunately, exposure of animals to diesel exhaust particulate matter alone, via inhalation, have not been carried out due to technical difficulties. Because there are insufficient data regarding possible interactions with the particulate matter fraction, it is not considered practical, at this time, to attempt to account separately for possible effects of the gaseous fraction. It should be noted, however, that because concentration of the gaseous component generally varies with the particulate matter phase, it is at least partially accounted for in the risk analysis.

Until recently, the particle adsorbed organics were considered to be the primary source of carcinogenicity in diesel exhaust. An early study, Kotin et al. (1955), reported that the organic compounds extracted from the surface of the diesel particles were capable of tumor induction. As detailed in Chapter 9, there is clear evidence that the organic constituents have the capacity to interact with DNA to give rise to mutations, chromosome alterations, and cell transformations, all well-established steps in the process of carcinogenesis. Furthermore, as noted in Chapter 2, the organic chemicals present include a variety of PAHs and nitroaromatics, many of which are known to be carcinogenic. These organics are eluted from the particles with a shorter half-time than clearance of the particles themselves from the deep lung (Sun et al., 1984). Following elution from the particles, the organics may diffuse into the alveolar spaces and be taken up by susceptible lung cells, although amounts might be limited by absorption into the bloodstream, metabolism by macrophages, etc.

The DNA adduct studies conducted by Bond et al. (1989) and detailed in Chapter 10 provided some evidence of a role for the organic moieties in tumor induction. For example, diesel exhaust exposure induced adduct formation at particulate matter concentrations of 3.5 $mg/m^3$. Carbon black having only traces of organics also induced increases in adducts, but only at concentrations three times as high (Bond et al., 1989). It is possible that the carbon black effect seen at the higher dose was macrophage mediated, whereas the adducts induced at lower doses of diesel exhaust were induced by organic constituents. If both

fractions induce adducts, then it is more likely that both are involved in the carcinogenic process.

Most of the adducts occurred in peripheral lung tissue where tumors are found. Bond et al. (1989) also reported a 60% increase in lung adduct levels of rats exposed to a diesel particle concentration of 8.1 $mg/m^3$, compared with no significant increases in the more tumor—resistant hamster and mouse lungs, lending further support to a link between adduct formation and subsequent tumor formation. It remains to be proven, however, whether the adducts induced are specific for the mutational changes responsible for induction of cancer.

Additional evidence suggesting a role for the organic phase was provided by Mumford et al. (1989). They reported greatly increased lung cancer mortality rates in Chinese communes burning so called "smoky coal" but not wood or smokeless coal, in unvented open pit fires used for heating and cooking. Particle concentrations ranged from 10 to 25 $mg/m^3$ in communes burning either smoky coal or wood, but PAH concentrations were five to six times greater in the air of communes burning smoky coal. Thus, cancer mortality correlated more closely with concentrations of PAHs than with particles. In the case of smokeless coal, both particle and PAH concentration were low. Demonstration of the carcinogenicity of coke oven emissions in humans (Lloyd, 1971) also provided evidence for a role by the organics, because coke oven particulate matter lacks an insoluble carbon core.

Unquestionably, the organic phase of diesel exhaust contains known carcinogenic compounds, most of which are aromatics. As can be seen in Table 3-5, however, the concentration of polycyclic aromatic compounds (PAHs) are quite low in diesel exhaust. For example, all of the PAHs measured by Tong and Karasek (1984) account for only 25 to 50 $\mu$g/mg of extract. If it is assumed that 20% of the particulate matter mass is composed of organic matter, then the total concentration of PAHs ranges from 5 to 10 $\mu$g/mg of particulate matter. Furthermore, many of the PAHs are not known to be carcinogenic. The concentration of the best known carcinogenic PAH (B[*a*]P) is present at concentrations of no more than 0.1 $\mu$g/mg particulate matter, based upon the Tong and Karasek (1984) data. It is unlikely that such low concentrations could be responsible for the degree of tumorigenic responses seen at the concentrations of diesel exposures employed. Further support of a minor role for organics is provided by the report that pyrolyzed pitch condensate, having about three orders of magnitude greater concentration of organics than diesel exhaust

1 particles, but no insoluble particle core, was only about as potent as diesel exhaust in the
2 induction of cancer (Heinrich et al., 1986a).
3 A hypothesis first proposed by Vostal (1986) is based on the belief that "diesel"
4 particles themselves induce lung cancer, probably through a secondary effect. In support of
5 this hypothesis are the observations that lung cancer can be induced by inhalation of so-called
6 "inert" particles such as titanium dioxide (Lee et al., 1986) and coal dust (Martin et al.,
7 1977) or by intratracheal instillation of activated carbon (Kawabata et al., 1986). Heinrich
8 (1990) reported that inhalation of carbon black particles, which are virtually devoid of
9 organics and are similar to the carbon core of diesel exhaust, also induced lung cancer in rats
10 and did so at concentrations comparable to those used in the diesel exhaust studies.
11 Mauderly et al. (1991, 1993) reported similar results. Finally, Kawabata et al. (1994)
12 induced lung tumors in rats intratracheally instilled with diesel exhaust particulate matter
13 from which the organic components had been extracted. The extracted particles were
14 effective at doses comparable to those for unextracted particles. A third possibility is that
15 both particles and organics are important for tumor induction because the interaction of the
16 two fractions may result in an enhanced effectiveness of the organics. Stenback et al.
17 (1976), for example, reported that intratracheal instillation of B[*a*]P adsorbed to particles is
18 much more effective than in its pure form.
19 The interaction of particles with organics could occur in several ways, although
20 evidence is still lacking. Adsorption to particles may result in more effective penetration of
21 the organics into alveolar regions, where clearance is slower. If the organics are condensed
22 onto particles, they are more likely to be taken up by macrophages, where possible partial
23 activation of the carcinogenic PAHs may occur. In fact, Bond et al. (1984) provided
24 evidence that alveolar macrophages from beagle dogs metabolized B[*a*]P coated on diesel
25 exhaust particles to proximate carcinogenic forms. The relatively slow sustained release of
26 organics from the particles may also provide a relatively constant supply of xenobiotics to the
27 target cells.
28 The alternative model differs from the LMS approach in that both the particle as well
29 as the particle adsorbed organics are assumed to have a role in the carcinogenesis process.
30 The usefulness of the alternative model is based upon its ability to compare estimated risks
31 after varying the coefficients for initiation and/or proliferation of organic and particulate

matter phases. For example, if the coefficient for initiation by the organic phase is reduced to zero, the risk is only reduced modestly (78% compared to the original model). On the other hand, if the coefficient for proliferation by particulate matter is increased to only 1.4 times that in the original model, the estimated risk nearly doubles. See Appendix C for details. Although cell proliferation appears to have a greater influence upon cancer induction in this model, this likelihood remains unproven since the coefficients for each parameter in the model cannot be accurately determined at this time. If data becomes available to accurately determine the proper coefficients, then it will be possible to account for the relative contribution of both the particle and vapor phases for cancer induction.

Until the coefficients in the alternative model are determined, or other means of partitioning risk among the components of diesel exhaust are developed, a unit risk based upon particulate matter and use of the linearized multistage model is considered to be the most reasonable. This conclusion was reached not only because of the uncertainty in determining coefficients in the alternate model, because of the demonstrated ability of pure carbon to induce lung tumors and because of the low concentration of known carcinogens in the organic.

Another important issue concerns extrapolation of tumor responses to ambient concentrations. As can be seen from Tables 11-2 through 11-4, significant increases in lung tumor incidences were not detected at the low exposure concentrations used in each of these studies. Based upon exposure rates, the data might be interpreted as evidence for a threshold in tumorigenic response. When the tumor responses were plotted in terms of modeled lung burden (Figure 11-3), however, a change in slope at the low doses was not apparent. In fact, for each of these studies, the data showed a very good statistical fit to a straight line. The effect of clearance rates can be more clearly seen in Figure 11-4, where modeled lung burdens in the three studies used for risk estimation are plotted against exposure rates. The lung burden/exposure rate ratios are much lower at doses not inducing clearance inhibition. Because the lung burdens were so small at the low exposure rates, detectable tumorigenic responses would not be predicted.

Although the relationship between lung particle burden and tumor response admittedly does not provide proof for the absence of a threshold, or a change in slope of the dose-response curve at low exposures, it also fails to provide evidence to the contrary.

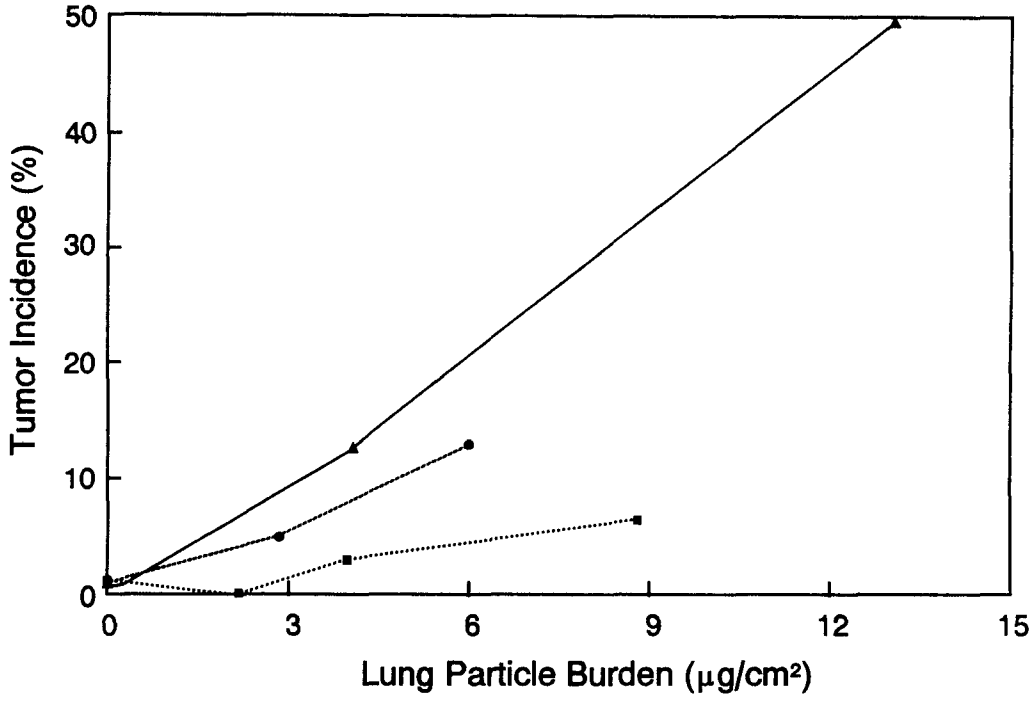


**Figure 11-3. Relationship between lung tumor incidence and modeled lung particle burden/unit of lung surface area using data from Brightwell et al. (1986), Ishinishi et al. (1986), and Mauderly et al. (1987).**

Nevertheless, since it appears that the primary means of cancer induction is macrophage mediated, the possibility of nonlinear responses at low concentrations exists. Unfortunately, information regarding mechanisms of action are inadequate. Based upon available data, lung injury appears likely to be induced by release of mediators from activated macrophages (e.g., reactive oxygen species, chemotactic factors, lysosomal hydrolases, other proteinases, prostaglandins, plasminogen activators, and growth factors. Oberdörster and Yu (1991) have outlined a hypothetical biological model, incorporating these factors, to explain the toxic and carcinogenic effects of particles.

The relationship between macrophage particle burden and function may be quite complex. The secretion of various mediators by individual macrophages may be proportional to particle load, may be disproportional with no threshold, or may have a threshold. The particles, moreover, are unevenly distributed among macrophages. Thus, some macrophages may be overloaded even at low exposure levels. Furthermore, it is uncertain if the target organ response to the mediators is linear or nonlinear at low doses. Because changes in the

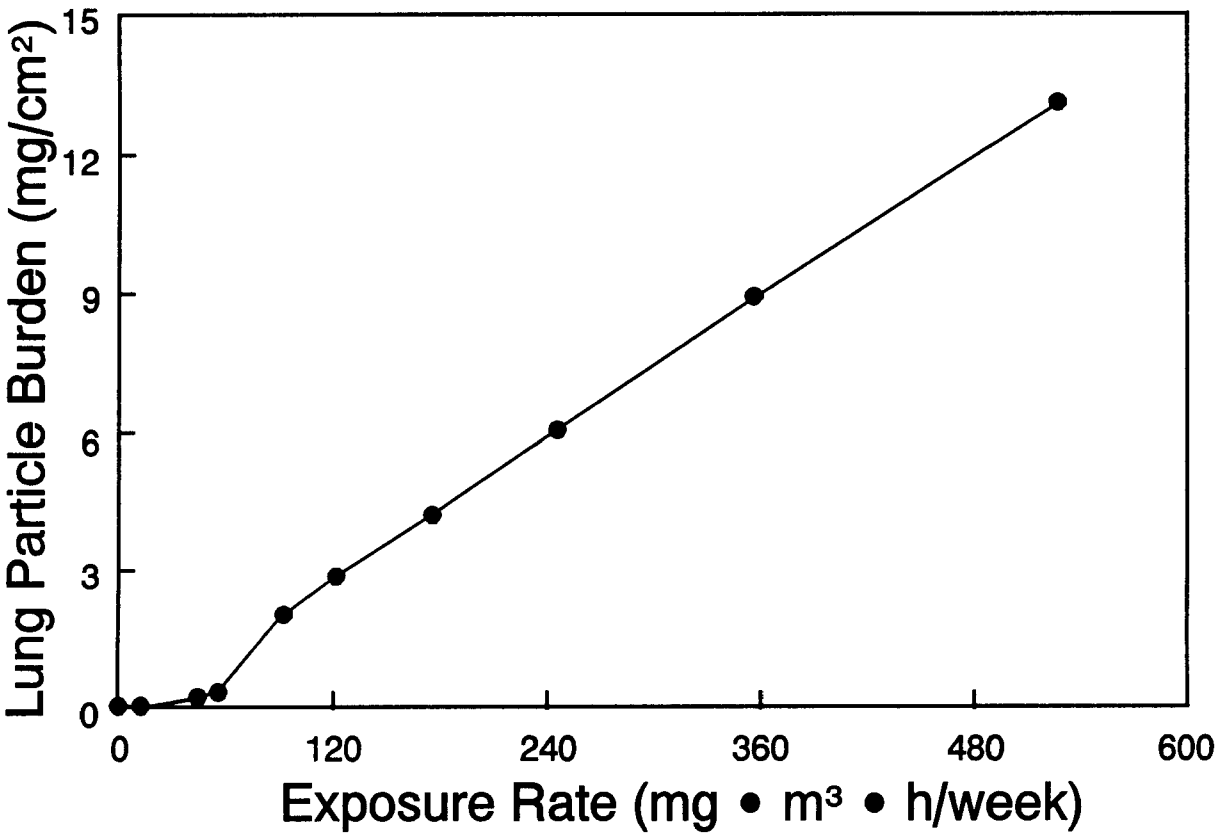


**Figure 11-4. Relationship between exposure rate and lung particle burden/unit of lung surface area using data from Brightwell et al. (1986), Ishinishi et al. (1986), and Mauderly et al. (1987).**

dose-response curve in the cancer bioassays cannot be detected when dose is converted to lung burden, and because data regarding release as well as effects of the supposed factors mediating cancer induction are inadequate, it is considered inappropriate, at this time, to attempt to adopt other than a linearized low-dose extrapolation model.

The LMS approach is also considered to be prudent and not likely to be overly conservative for a number of other reasons as well. Even though the organics may not have a major role in tumor induction, they cannot be totally discounted. Furthermore, any initiating effects of organics are likely to be a straight line function of dose even at very low doses. Nondiesel particulate matter is present in ambient air at widely varying concentrations. Although it cannot be assumed that all particles have similar cancer inducing properties, nevertheless ambient particulate matter does contribute to macrophage particle

burdens. There is also evidence that particle clearance in humans is slowed in smokers (Bohning et al., 1982) and in individuals with respiratory disease (Cohen et al., 1979; Freedman et al., 1988). As a result, there may be a large population already at the threshold, or above, for lung particle overload. Because the lung burden model assumes normal clearance at ambient particle concentrations, individuals with impaired clearance may be near the upper bound estimates of risk. Bond et al. (1989) reported that levels of DNA adducts were greater in the lungs of monkeys than in the lungs of rats exposed to the same concentration of diesel exhaust, suggesting the possibility of increased sensitivity in a species genetically more similar to humans. Finally, higher, although admittedly highly uncertain risk estimates, derived from epidemiology data suggest that a conservative approach would be prudent when extrapolating risk from animal data.

Attempts have been made to determine what characteristics of insoluble particles are responsible their effects. As can be seen in Table 11-6, the concentrations of particles required to induce tumors varied widely with particle type. Oberdörster and Yu (1991) plotted tumor response against various parameters of the retained particles (i.e., volume, weight, particle numbers, particle surface area, etc.). They determined that only the surface area of the retained particles correlated well with observed lung tumors in the rat studies. Assuming a particle surface area of 90 $m^2/g$ for diesel particles after the elution of organics (Pierson and Brachaczek, 1976), the diesel cancer data fit a surface area-tumor incidence plot quite well.

Although it is reasonable to assume that particle surface plays an important role in the toxicity of inhaled insoluble particulate matter, other particle characteristics cannot be ruled out. In a recent study by Sagai et al. (1993), for example, the toxicity of diesel particulate matter was claimed to be also related to the presence of oxygen radicals on the particle surface. In any case, the cancer potency estimates for diesel exhaust should not be used to estimate risk from exposure to other types of insoluble particulate matter found in the ambient environment, because of their likely differing characteristics. In the present assessment it is assumed that experimental animals and humans are exposed to the same types of particles, so particle characteristics such as surface area do not need to be adjusted for.

**TABLE 11-6. CANCER STUDIES WITH RATS EXPOSED TO RELATIVELY CHEMICALLY INERT DUSTS AT EXPOSURE CONCENTRATIONS OF SEVERAL MILLIGRAMS PER CUBIC METER OR ABOVE**

| Reference | Particle Type | Concentration ($mg/m^3$) | Duration | Cancer |
|---|---|---|---|---|
| Martin et al. (1977) | Coal dust | 200 | Not reported | Yes |
| Lee et al. (1986) | $TiO_2$ | 10-250 | 2 years | Yes (250 $mg/m^3$) |
| Heinrich (1993) | $TiO_2$[a] | 11.3 | 2 years | Yes |
| Muhle et al. (1991) | Toner particles | 1-16 | 2 years | No |
| Heinrich (1990) | Carbon black | 6 | 10-20 mo | Yes |
| Mauderly et al. (1991) | Carbon black | 6.5 | 24 mo | Yes |

[a]Ultrafine particles.

### 11.5.4.2 Evaluation of Animal-Based Risk Estimates Against Human Experience

It is of interest to evaluate the reasonableness of the bioassay-based risk estimates (which range from 1.6 to $7.1 \times 10^{-5}$ with a geometric mean of $3.4 \times 10^{-5}$) against human experience. Three sets of data offer such an opportunity: the Harris (1983) analysis of an epidemiological study conducted on London Tranport Authority workers by Waller (1981) and Garshick et al. (1987, 1988) on U.S. railroad workers. Although the human data are considered to be unsuitable as a basis for calculating a unit risk (mainly due to the lack of reliable exposure information), they can be used to evaluate the reasonableness of the animal-based unit risk estimates.

Several attempts were made to estimate potential cancer risk due to exposure to diesel exhaust on the basis of epidemiological data. Based on the London transport study, Harris (1983) estimated that the increase in relative risk of lung cancer associated with 1 $\mu g/m^3$/year of diesel exhaust exposure is $1.2 \times 10^{-4}$ with a 95% upper bound of $4.8 \times 10^{-4}$. As a general practice when using data from a nonpositive epidemiological study to estimate cancer risk, the unit risk is based upon the 95% upper bound. The resultant unit risk estimate was equal to $2 \times 10^{-3}$ ($4.8 \times 10^{-4} \times 70$ $\mu g/m^3$-years $\times$ 0.06), which is about 60-fold higher than the mean animal-based unit risk estimate of $3.4 \times 10^{-5}$ or about 30-fold greater than the upper end of the range of animal-based unit risk estimates, $7.1 \times 10^{-5}$. The animal-based

risk estimate is not considered to be inconsistent with this human-based estimate because $2 \times 10^{-3}$ is an upper bound estimate from a nonpositive epidemiological study.

More recently, McClellan et al. (1989) reported risk estimates based on the Garshick et al. (1987) study in which lung cancer in railroad workers was evaluated. Assuming exposure concentrations of 500 and 125 $\mu g/m^3$, 95% upper bounds for lifetime cancer risk per micrograms per cubic meter were estimated to be $6 \times 10^{-4}$ and $2 \times 10^{-3}$, respectively. The smaller value of these two unit risk estimates is only about an order of magnitude greater than the animal-based ones.

An epidemiological study that is potentially more suitable for quantitative risk assessment was reported by Garshick et al. (1988). This study included a large number of subjects and a small but significant increase in lung cancer mortality risk for some subcohorts. Recently, the EPA has supported an effort to derive a unit risk estimate using Garshick et al. (1988) and exposure data estimated by Woskie et al. (1988a,b; see Appendix B for details). The data were analyzed in a variety of different ways by using relative risk and absolute risk dose-response models and by classifying individuals into various exposure categories according to job classification, duration of employment, age, different exposure markers, etc. Even though at least 50 different analyses were carried out, an adequate dose-response relationship could not be obtained.

The lack of a dose-response relationship is not totally unexpected, given the smallness of mortality rate increase observed in the Garshick study, and great uncertainty associated with the exposure estimates. The exposure data used in these studies came from air samples collected during a limited time period (between 1981 and 1983) at four small railroads operating in a limited geographical area (northern United States). Measured concentrations were of respirable particulate matter rather than diesel exhaust per se, and these measured concentrations were adjusted to produce markers of diesel exposure. These data were used to estimate exposures to diesel exhausts that occurred among railroad workers throughout the United States as much as 30 or more years ago. Diesel equipment and working conditions have changed since the 1940s when the use of large numbers of diesel engines first began. (Woskie et al. (1988b) described anecdotal reports of smoky working conditions in the diesel repair shops during the 1950s and 1960s. They also reported that limited data available on

nitrogen oxide levels during these periods provide qualitative support for the likelihood of higher levels of diesel exhaust concentration in the early years of dieselization. By the time samples were collected for this study, these smoky conditions would have been largely mitigated through improved ventilation and the advent of less smoky engines.

Based on Garshick's study, the relative risks of lung cancer death in exposed versus unexposed railroad workers (classified into five subcohorts by ages in 1959) ranged from 0.96 with 95% confidence interval (0.74, 1.3) to 1.49 with 95% confidence interval (1.1, 1.9) The highest relative risk of 1.49 was observed from the workers who were 40 to 49 years old in 1959. This highest relative risk was used to evalute the reasonableness of the animal-based unit risk estimate. It was assumed that this subcohort of workers was exposed to diesel exhaust 8 h/day, 5 days/week, from age 35 to 65. The background lung cancer mortality rate for this subcohort is estimated to be 0.038 ($0.63 \times 0.06$), based on the fact that the unexposed workers in the same age group had a relative risk of 0.63, and that the corresponding lifetime lung cancer mortality rate in the general U.S. white male population is about 0.06. If the lung cancer risk due to 1 $\mu g/m^3$ is assumed to be $3.4 \times 10^{-5}$, it would imply that the diesel concentration in their work environment was at least 400 $\mu g/m^3$. This is calculated as follows: The risk due to 1 $\mu g/cm^2$ of particle lung burden is 0.017 (which results in a unit risk of $3.4 \times 10^{-5}$). To have a lower bound relative risk of 1.1, one would need a lung burden (d) $ug/cm^2$ that satisfies the relationship $0.1 \times 0.038 = 0.017d$ (i.e., $d = 0.22$ $\mu g/cm^2$, which is equivalent to an air concentration of 0.4 $mg/m^3$ by using the dosimetry model of Yu et al., 1991). If the highest unit risk estimate $7.1 \times 10^{-5}$ derived from the Brightwell et al. (1986) study is used, then it would require at least 0.2 $mg/m^3$ of diesel exhaust concentration to observe a statistically significant elevation of lung cancer mortality rate in this study. These air concentrations appear reasonable in light of working conditions described by Woskie et al. (1988a).

Although the animal-based risk estimates are lower than those derived from human data, they are not inconsistent for the following reasons:

(1) The human-base risk numbers are not derived from all available data; the numbers are based only on a subset of data that showed the highest response. Therefore, the resultant risk estimates are expected to be higher than if the whole data set is used.

(2) When a single data point (i.e., an overall relative risk and an averaged exposure concentration) is used in the calculations, the resultant potency slope always results in an overestimate of the slope factor if the dose-response relationship is not a straight line over all exposure concentrations. To see this, one can assume (as an example) that the cancer response follows a simple multistage model $P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2)]$. The relative risk at a concentration "d" is $R(d) = P(d)/P(0)$. Using the mathematical expression of R(d), it is easy to demonstrate that the slope factor calculated by $[R(d) - 1]P(0)/d$ is always greater at higher doses (which include the averged concentration used in the risk calculation) than at low doses where the dose-response function is dominated by $q_1$. A similar (but not identical) concern of using averaged data has long been recognized by epidemiologists who are concerned about the fact that ecologic or group-level associations are not necessarily consistent with those measured at the individual level (see Greenland and Robins, 1994a,b; Piantadosi, 1994; Cohen, 1994).

(3) There is evidence that various occupational groups were exposed to considerably higher concentrations of diesel exhaust in the past than presently. For example, particle concentration in a Finnish roundhouse was reported to average 2 $mg/m^2$ (Heino et. al., 1978). This will result in greater lung burdens than predicted from present exposures and, thereby, greater unit risk estimates.

(4) It is a reasonable assumption that the occupationally exposed groups on which the unit risks were based were also exposed to greater nondiesel dust concentrations in their earlier employment. This would again result in increased lung burdens and possibly contribute to an overestimate of cancer risk.

#### 11.5.4.3 Reasonableness of the Unit Risk Estimate

The dose-response analysis contained in this assessment of cancer risk has the following positive features.

(1) The estimates are based upon several well designed, long-term animal studies.

(2) Epidemiology studies indicate that humans are susceptible to tumor induction by inhalation of diesel exhaust.

(3) Dosimetry modeling, especially the portion accounting for high-dose inhibition of particle clearance, has allowed more accurate extrapolation of dose from animals to humans.

(4) Dose is based upon actual concentration of particulate matter per unit lung surface area.

(5) Use of an alternative model that attempted to account for initiation by the organic fraction did not result in an appreciable change in the unit risk estimate.

Nevertheless, a number of uncertainties remain, the more significant ones of which are listed below:

(1) In any species extrapolation there is the possibility of an inherent difference in sensitivity to the agent being assessed.

(2) The selection of the inorganic particulate matter fraction to base risk on. Although evidence strongly supports the likelihood that particles are the major cause of lung cancer induction by diesel exhaust, the organic constituents probably do contribute at least minimally to the carcinogenic response. Because low-dose extrapolation of organic matter lung burden is less influenced by lung overload than particles, the unit risk estimate may differ somewhat if risk is base upon the organic fraction. Nevertheless, a unit risk estimate based upon the organic fraction using the same data sets, LMS and dosimetry model would differ by less than 25% from the particle based one. Furthermore, use of the alternative model, incorporating possible organic effects, as well as particle effects also failed to provide a significantly different unit risk estimate.

(3) A departure from most EPA cancer risk assessments (Federal Register, 1986) is the assumption of equivalent sensitivity across species based upon concentration per unit of lung surface area. Although it is reasonable to assume that slower metabolism by human lung epithelial cells is offset by slower production and release of harmful factors, this assumption is still unproven.

(4) Use of linearized low-dose extrapolation methods. It is still uncertain whether macrophages secrete mediators thought to induce cancer in lung epithelial cells at particles burdens less than those necessary to induce inhibition of clearance. Even if macrophages are activated at low particle burdens, it is uncertain if responses of epithelial cells are linear at very low concentration. This area is considered to be a primary research need.

(5) Heavy smokers and individuals exposed occupationally to dusty environments may have lung particle burdens at or near threshold levels for inhibition of particle clearance. The dose-response curve in such cases may be steeper than predicted by extrapolation modeling, which is based upon normal clearance rates.

Overall, the unit risk estimate is considered to be reasonable because it is based upon well-designed and conducted chronic inhalation experiments, human doses are estimated using detailed modeling techniques, and results agree within an order of magnitude with unit risks developed using a variety of approaches. Nevertheless, there is still a considerable possibility that risk may be overestimated for some populations and underestimated in others.

## 11.6 SUMMARY AND CONCLUSIONS

As a result of limited evidence from epidemiological data, supported by adequate evidence for carcinogenicity of diesel engine emissions in animal studies, as well as positive evidence for mutagenicity, it was concluded that diesel engine emissions best fit into cancer weight-of-evidence Category B1. Diesel engine emissions are thus considered to be probable human carcinogens. This is in agreement with a 2A classification by International Agency for Research on Cancer.

Using a dosimetry model that accounted for animal-to-human differences in lung deposition efficiency, lung particle clearance rates, lung surface area, ventilation, metabolic rate, as well as elution rates of organic chemicals from the particle surface, equivalent human doses were calculated on the basis of particle concentration per unit lung surface area. Following dosimetric adjustment, risk estimates were derived using a linearized multistage model. A unit risk estimate of $3.4 \times 10^{-5}$ (the upper 95% bound of the cancer risk from lifetime exposure to 1 $\mu g/m^3$ diesel particulate matter) is recommended. This estimate is based on the geometric mean of estimates derived from three separate animal bioassays using Fischer 344 rats.

This unit risk estimate should not be used to evaluate the cancer risk of other types of particulate matter present in the ambient air. These particles may have differing solubilities, surface areas, presence of free radicals, or other properties which may greatly affect cancer potency.

# REFERENCES


Albert, R. E.; Chen, C. (1986) U.S. EPA diesel studies on inhalation hazards. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenicity and mutagenicity of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 411-419. (Developments in toxicology and environmental science: v. 13).

Albert, R. E.; Lewtas, J.; Nesnow, S.; Thorslund, T. W.; Anderson, E. (1983) Comparative potency method for cancer risk assessment: application to diesel particulate emissions. Risk Anal. 3: 101-117.

Benhamou, S.; Benhamou, E.; Flamant, R. (1988) Occupational risk factors of lung cancer in a French case-control study. Br. J. Ind. Med. 45: 231-233.

Boffetta, P.; Stellman, S. D. (1988) Association between diesel exhaust exposure and multiple myeloma: an example of confounding. Prev. Med. 17: 236-237.

Bohning, D. E.; Atkins, H. L.; Cohn, S. H. (1982) Long-term particle clearance in man: normal and impaired. In: Walton, W. H., ed. Inhaled particles V: proceedings of an international symposium; September 1980; Cardiff, Wales. Ann. Occup. Hyg. 26: 259-271.

Bond, J. A.; Butler, M. M.; Medinsky, M. A.; Muggenburg, B. A.; McClellan, R. O. (1984) Dog pulmonary macrophage metabolism of free and particle-associated [$^{14}$C]benzo[a]pyrene. J. Toxicol. Environ. Health 14: 181-189.

Bond, J. A.; Harkema, J. R.; Henderson, R. F.; Mauderly, J. L.; McClellan, R. O.; Wolff, R. K. (1989) Molecular dosimetry of inhaled diesel exhaust. In: Mohr, U.; Bates, D. V.; Dungworth, D. L.; Lee, P. N.; McClellan, R. O.; Roe, F. J. C., eds. Assessment of inhalation hazards: integration and extrapolation using diverse data: [papers from the 2nd international inhalation symposium]; 1989; Hannover, FRG. Berlin, Federal Republic of Germany: Springer-Verlag; pp. 315-324.

Brightwell, J.; Fouillet, X.; Cassano-Zoppi, A.-L.; Gatz, R.; Duchosal, F. (1986) Neoplastic and functional changes in rodents after chronic inhalation of engine exhaust emissions. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 471-485. (Developments in toxicology and environmental science: v. 13).

Chan, T. L.; Lee, P. S.; Hering, W. E. (1981) Deposition and clearance of inhaled diesel exhaust particles in the respiratory tract of Fischer rats. J. Appl. Toxicol. 1: 77-82.

Cohen, B. L. (1994) Invited commentary: in defense of ecologic studies for testing a linear-no threshold theory. Am. J. Epidemiol. 139: 765-768.

Cohen, D.; Arai, S. F.; Brain, J. D. (1979) Smoking impairs long-term dust clearance from the lung. Science (Washington, DC) 204: 514-517.

Crump, K. S.; Lambert, T.; Chen, C. (1991) Assessment of risk from exposure to diesel engine emissions. Research Triangle Park, NC: U.S. Environmental Protection Agency; contract no. 68-02-4601.

Cuddihy, R. G.; McClellan, R. O. (1983) Evaluating lung cancer risks from exposures to diesel engine exhaust. Risk Anal. 3: 119-124.

Cuddihy, R. G.; Griffith, W. C.; Clark, C. R.; McClellan, R. O. (1981) Potential health and environmental effects of light duty diesel vehicles II. Albuquerque, NM: Lovelace Biomedical and Environmental Research Institute, Inhalation Toxicology Research Institute; report no. LMF-89.

Cuddihy, R. G.; Griffith, W. C.; McClellan, R. O. (1984) Health risks from light-duty diesel vehicles. Environ. Sci. Technol. 18: 14A-21A.

Damber, L. A.; Larsson, L. G. (1987) Occupation and male lung cancer: a case-control study in northern Sweden. Br. J. Ind. Med. 44: 446-453.

Edling, C.; Anjou, C.-G.; Axelson, O.; Kling, H. (1987) Mortality among personnel exposed to diesel exhaust. Int. Arch. Occup. Environ. Health 59: 559-565.

Federal Register. (1986) Guidelines for carcinogen risk assessment. F. R. (September 24) 51: 33992-34003.

Freedman, A. P.; Robinson, S. E.; Street, M. R. (1988) Magnetopneumographic study of human alveolar clearance in health and disease. Ann. Occup. Hyg. 32(suppl. 1): 809-820.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1987) A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 135: 1242-1248.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1988) A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 137: 820-825.

Greenland, S.; Robins, J. (1994a) Invited commentary: ecologic studies—biases, misconceptions, and counterexamples. Am. J. Epidemiol. 139: 747-760.

Greenland, S.; Robins, J. (1994b) Accepting the limits of ecologic studies: Drs. Greenland and Robins reply to Drs. Piantadosi and Cohen. Am. J. Epidemiol. 139: 769-771.

Griffis, L. C.; Wolff, R. K.; Henderson, R. F.; Griffith, W. C.; Mokler, B. V.; McClellan, R. O. (1983) Clearance of diesel soot particles from rat lung after a subchronic diesel exhaust exposure. Fundam. Appl. Toxicol. 3: 99-103.

Grimmer, G.; Brune, H.; Deutsch-Wenzel, R.; Dettbarn, G.; Jacob, J.; Naujack, K.-W.; 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. (Shannon, Irel.) 37: 173-180.

Hall, N. E. L.; Wynder, E. L. (1984) Diesel exhaust exposure and lung cancer: a case-control study. Environ. Res. 34: 77-86.

Harris, J. E. (1983) Diesel emissions and lung cancer. Risk Anal. 3: 83-100.

Hayes, R. B.; Thomas, T.; Silverman, D. T.; Vineis, P.; Blot, W. J.; Mason, T. J.; Pickle, L. W.; Correa, P.; Fontham, E. T. H.; Schoenberg, J. B. (1989) Lung cancer in motor exhaust-related occupations. Am. J. Ind. Med. 16: 685-695.

Heino, M.; Ketola, R.; Makela, P.; Makinen, R.; Niemala, R.; Starck, J.; Partanen, T. (1978) Work conditions and health of locomotive engineers: noise, vibration, thermal climate, diesel exhaust constituents, ergonomics. Scand. J. Work Environ. Health 4: 3-14.

Heinrich, U. (1990) Results of long-term inhalation exposure of rats to carbon black "Printex 90" [letter to Dr. Lester D. Grant]. Presented at: U.S. Environmental Protection Agency peer review workshop on the Health Assessment Document for Diesel Emissions; July; Research Triangle Park, NC.

Heinrich, U. (1993) Carcinogenic effects of solid particles. In: Toxicity and carcinogenicity of solid particles in the respiratory tract—proceedings of the 4th international inhalation symposium; Hannover. pp. 43.

Heinrich, U.; Peters, L.; Funcke, W.; Pott, F.; Mohr, U.; Stöber, W. (1982) Investigation of toxic and carcinogenic effects of diesel exhaust in long-term inhalation exposure of rodents. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 225-242. (Developments in toxicology and environmental science: v. 10).

Heinrich, U.; Pott, F.; Rittinghausen, S. (1986a) Comparison of chronic inhalation effects in rodents after long-term exposure to either coal oven flue gas mixed with pyrolized pitch or diesel engine exhaust. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite syposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 441-457. (Developments in toxicology and environmental science: v. 13).

Heinrich, U.; Muhle, H.; Takenaka, S.; Ernst, H.; Fuhst, R.; Mohr, U.; Pott, F.; Stöber, W. (1986b) Chronic effects on the respiratory tract of hamsters, mice, and rats after long-term inhalation of high concentrations of filtered and unfiltered diesel engine emissions. J. Appl. Toxicol. 6: 383-395.

Howe, G. R.; Fraser, D.; Lindsay, J.; Presnal, B.; Yu, S. Z. (1983) Cancer mortality (1965-77) in relation to diesel fume and coal exposure in a cohort of retired railway workers. JNCI J. Natl. Cancer Inst. 70: 1015-1019.

Huff, J. E.; Haseman, J. K.; DeMarini, D. M.; Eustis, S.; Maronpot, R. R.; Peters, A. C.; Persing, R. L.; Chrisp, C. E.; Jacobs, A. C. (1989) Multiple site carcinogenicity of benzene in Fischer 344 rats and $B6C3F_1$ mice. Environ. Health Perspect. 82: 125-163.

Huisingh, J.; Bradow, R.; Jungers, R.; Claxton, L.; Zweidinger, R.; Tejada, S.; Bumgarner, J.; Duffield, F.; Waters, M.; Simmon, V. F.; Hare, C.; Rodriguez, C.; Snow, L. (1978) Application of bioassay to the characterization of diesel particle emissions. In: Waters, M. D.; Nesnow, S.; Huisingh, J. L.; Sandhu, S. S.; Claxton, L., eds. Application of short-term bioassays in the fractionation and analysis of complex environmental mixtures: [proceedings of a symposium; February; Williamsburg, VA]. New York, NY: Plenum Press; pp. 383-418. (Hollaender, A.; Probstein, F.; Welch, B. L., eds. Environmental science research: v. 15).

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

Ishinishi, N.; Kuwabara, N.; Nagase, S.; Suzuki, T.; Ishiwata, S.; Kohno, T. (1986) Long-term inhalation studies on effects of exhaust from heavy and light duty diesel engines on F344 rats. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 329-348. (Developments in toxicology and environmental science: v. 13).

Ishinishi, N.; Inamasu, T.; Hisanaga, A.; Tanaka, A.; Hirata, M.; Ohyama, S. (1988) Intratracheal instillation study of diesel particulate extracts in hamsters. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 209-216.

Ishinishi, N.; Kuwabara, N.; Takaki, Y.; Nagase, S.; Suzuki, T.; Nakajima, T.; Maejima, K.; Kato, A.; Nakamura, M. (1988) Long-term inhalation experiments on diesel exhaust. In: Diesel exhaust and health risks: results of the HERP studies. Tsukuba, Ibaraki, Japan: Japan Automobile Research Institute, Inc., Research Committee for HERP Studies; pp. 11-84.

Iwai, K.; Udagawa, T.; Yamagishi, M.; Yamada, H. (1986) Long-term inhalation studies of diesel exhaust on F344 SPF rats. Incidence of lung cancer and lymphoma. In: Ishinishi, N.; Koizumi, A.; McClellan, R.O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 349-360. (Developments in toxicology and environmental science: v. 13).

Jones, K. H. (1993) Diesel truck emissions, an unrecognized source of PCDD/PCDF exposure in the United States. Risk Anal. 13: 245-252.

Karagianes, M. T.; Palmer, R. F.; Busch, R. H. (1981) Effects of inhaled diesel emissions and coal dust in rats. Am. Ind. Hyg. Assoc. J. 42: 382-391.

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. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 213-222. (Developments in toxicologial and environmental science: v. 13).

Kawabata, Y.; Udagawa, T.; Higuchi, K.; Yamada, H.; Iwai, K. (1994) Early one-year exposure to diesel exhaust causes lung cancer in rats. In: Mohr, U.; Dungworth, D. L.; Mauderly, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 429-431.

Kotin, P.; Falk, H. L.; Thomas, M. (1955) Aromatic hydrocarbons: III. presence in the particulate phase of diesel-engine exhausts and the carcinogenicity of exhaust extracts. AMA Arch. Ind. Health 11: 113-120.

Krewski, D.; Crump, K. S.; Farmer, J.; Gaylor, D. W.; Howe, R.; Portier, C.; Salsburg, D.; Sielken, R. L.; Van Ryzin, J. (1983) A comparison of statistical methods for low dose extrapolation utilizing time-to-tumour data. Fundam. Appl. Toxicol. 3: 140-160.

Kuschner, M. (1968) The J. Burns Amberson lecture: the causes of lung cancer. Am. Rev. Respir. Dis. 98: 573-590.

Lee, K. P.; Henry, N. W., III; Trochimowicz, H. J.; Reinhardt, C. F. (1986) Pulmonary response to impaired lung clearance in rats following excessive $TiO_2$ dust deposition. Environ. Res. 41: 144-167.

Lerchen, M. L.; Wiggins, C. L.; Samet, J. M. (1987) Lung cancer and occupation in New Mexico. JNCI J. Natl. Cancer Inst. 79: 639-645.

Lloyd, J. W. (1971) Long-term mortality study of steelworkers: V. respiratory cancer in coke plant workers. J. Occup. Med. 13: 53-68.

Marklund, S.; Andersson, R.; Tysklind, M.; Rappe, C.; Egebäck, K.-E.; Björkman, E.; Grigoriadis, V. (1990) Emissions of PCDDs and PCDFs in gasoline and diesel fueled cars. Chemosphere 20: 553-561.

Martin, J. C.; Daniel, H.; Le Bouffant, L. (1977) Short- and long-term experimental study of the toxicity of coal mine dust and some of its constituents. In: Walton, W. H., ed. Inhaled particles IV, part 1: proceedings of an international symposium; September 1975; Edinburgh, United Kingdom. Oxford, United Kingdom: Pergamon Press; pp. 361-371.

Mauderly, J. L.; Jones, R. K.; Griffith, W. C.; Henderson, R. F.; McClellan, R. O. (1987) Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam. Appl. Toxicol. 9: 208-221.

Mauderly, J. L.; Snipes, M. B.; Barr, E. B.; Bechtold, W. E.; Henderson, R. F.; Mitchell, C. E.; Nikula, K. J.; Thomassen, D. G. (1991) Influence of particle-associated organic compounds on carcinogenicity of diesel exhaust. Presented at: eighth Health Effects Institute annual conference; April; Colorado Springs, CO. Cambridge, MA: Health Effects Institute.

Mauderly, J. L.; Henderson, R. F.; Nikula, K. J.; Snipes, M. B. (1993) Non-cancer effects of chronic inhalation exposure of animals to solid particles. In: Toxic and carcinogenic effects of solid particles in the respiratory tract—proceedings of the 4th international inhalation symposium; March; Hannover, Germany. p. 42.

McClellan, R. O. (1986) Health effects of diesel exhaust: a case study in risk assessment. Am. Ind. Hyg. Assoc. J. 47: 1-13.

McClellan, R. O.; Cuddihy, R. G.; Griffith, W. C.; Mauderly, J. L. (1989) Integrating diverse data sets to assess the risks of airborne pollutants. In: Mohr, U.; Bates, D. V.; Dungworth, D. L.; Lee, P. N.; McClellan, R. O.; Roe, F. J. C., eds. Assessment of inhalation hazards: integration and extrapolation using diverse data: [papers from the 2nd international inhalation symposium]; 1989; Hannover, FRG. Berlin, Federal Republic of Germany: Springer-Verlag; pp. 3-22.

Moolgavkar, S. H.; Knudson, A. G., Jr. (1981) Mutation and cancer: a model for human carcinogens. JNCI J. Natl. Cancer Inst. 66: 1037-1052.

Morrow, P. E. (1988) Possible mechanisms to explain dust overloading of the lungs. Fundam. Appl. Toxicol. 10: 369-384.

Muhle, H.; Bellmann, B.; Creutzenberg, O.; Dasenbrock, C.; Ernst, H.; Kilpper, R.; MacKenzie, J. C.; Morrow, P.; Mohr, U.; Takenaka, S.; Mermelstein, R. (1991) Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam. Appl. Toxicol. 17: 280-299.

Mumford, J. L.; Chapman, R. S.; Harris, D. B. (1989) Indoor air exposure to coal and wood combustion emissions associated with a high lung cancer rate in Xuan Wei, China. Environ. Int. 15: 315-320.

National Council on Radiation Protection and Measurements. (1980) Influence of dose and its distribution in time on dose-response relationships for low-LET radiations: recommendations of the National Council on Radiation Protection and Measurements. Washington, DC: National Council on Radiation Protection and Measurement; NCRP report no. 64.

Nesnow, S.; Evans, C.; Stead, A.; Creason, J.; Slaga, T. J.; Triplett, L. L. (1982) Skin carcinogenesis studies of emission extracts. In: Lewtas, J., ed. Toxicological effects of emissions from diesel engines: proceedings of the Environmental Protection Agency diesel emissions symposium; October 1981; Raleigh, NC. New York, NY: Elsevier Biomedical; pp. 295-320. (Developments in toxicology and environmental science: v. 10).

Nikula, K. J.; Snipes, M. B.; Barr, E. B.; Griffith, W. C.; Henderson, R. F.; Mauderly, J. L. (1994) Influence of particle-associated organic compounds on the carcinogenicity of diesel exhaust. In: Mohr, U.; Dungworth, D. L.; Mauderly, J. L.; Oberdörster, G., eds. Toxic and carcinogenic effects of solid particles in the respiratory tract: [proceedings of the 4th international inhalation symposium]; March 1993; Hannover, Germany. Washington, DC: International Life Sciences Institute Press; pp. 565-568.

Oberdörster, G.; Yu, C. P. (1991) The carcinogenic potential of inhaled diesel exhaust: a particle effect? J. Aerosol Sci. 21(suppl. 1): S397-S401.

Oehme, M.; Larssen, S.; Brevik, E. M. (1991) Emission factors of PCDD and PCDF for road vehicles obtained by tunnel experiment. Chemosphere 23: 1699-1708.

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

Piantadosi, S. (1994) Invited commentary: ecologic biases. Am. J. Epidemiol. 139: 761-764.

Pierson, W. R.; Brachaczek, W. W. (1976) Particulate matter associated with vehicles on the road. Warrendale, PA: Society of Automotive Engineers; SAE technical paper no. 760039. SAE Trans. 85: 209-227.

Portier, C.; Hedges, J. C.; Hoel, D. G. (1986) Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. Cancer Res. 46: 4372-4378.

Pott, F.; Heinrich, U. (1987) Dieselmotorabgas und Lungenk auf die Gefährdung des Menschen. Umwelthygiene (Düsseldorf) 19: 130-167.

Raffle, P. A. B. (1957) The health of the worker. Br. J. Ind. Med. 14: 73-80.

Rinsky, R. A.; Young, R. J.; Smith, A. B. (1981) Leukemia in benzene workers. Am. J. Ind. Med. 2: 217-245.

Rushton, L.; Alderson, M. R.; Nagarajah, C. R. (1983) Epidemiological survey of maintenance workers in London Transport Executive bus garages at Chiswick Works. Br. J. Ind. Med. 40: 340-345.

Saffiotti, U. et al. (1988) Multifactorial hamster respiratory carcinogenesis: oxide, pyrene and N-methyl-N-nitrosourea (MNU). Presented at: Biology, toxicology and carcinogenesis of respiratory epithelium; November; Albuquerque, NM.

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 Radical Biol. Med. 14: 37-47.

Sanders, C. (1989) [Personal communication].

Schuetzle, D.; Frazier, J. A. (1986) Factors influencing the emission of vapor and particulate phase components from diesel engines. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 41-63. (Developments in toxicology and environmental science: v. 13).

Schum, M.; Yeh, H.-C. (1980) Theoretical evaluation of aerosol deposition in anatomical models of mammalian lung airways. Bull. Math. Biol. 42: 1-15.

Smith, L. R. (1989) Characterization of exhaust emissions from trap-equipped light-duty diesels [final report]. Sacramento, CA: California Air Resources Board; contract no. A5-159-32.

Smith, R. A.; Stayner, L. (1990) An exploratory assessment of the risk of lung cancer associated with exposure to diesel exhaust based on a study with rats [final report]. Cincinnati, OH: National Institute for Occupational Safety and Health, Division of Standards Development and Technology Transfer.

Steenland, N. K.; Silverman, D. T.; Hornung, R. W. (1990) Case-control study of lung cancer and truck driving in the Teamsters Union. Am. J. Public Health 80: 670-674.

Stenbäck, F.; Rowland, J.; Sellakumar, A. (1976) Carcinogenicity of pyrene and dusts in the hamster lung (instilled intratracheally with titanium oxide, aluminum oxide, carbon and ferric oxide). Oncology 33: 29-34.

Stöber, W. (1986) Experimental induction of tumors in hamsters, mice and rats after long-term inhalation of filtered and unfiltered diesel engine exhaust. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 421-439. (Developments in toxicology and environmental science: v. 13).

Strom, K. (1987) Presented at: HEI diesel symposium; February; Seabrooke Island, SC.

Sun, J. D.; Wolff, R. K.; Kanapilly, G. M.; McClellan, R. O. (1984) Lung retention and metabolic fate of inhaled benzo[*a*]pyrene associated with diesel exhaust particles. Toxicol. Appl. Pharmacol. 73: 48-59.

Swenberg, J. A.; Kerns, W. D.; Mitchell, R. I.; Gralla, E. J.; Pavkov, K. L. (1980) Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. Cancer Res. 40: 3398-3401.

Tong, H. Y.; Karasek, F. W. (1984) Quantitation of polycyclic aromatic hydrocarbons in diesel exhaust particulate matter by high-performance liquid chromatography fractionation and high-resolution gas chromatography. Anal. Chem. 56: 2129-2134.

Vostal, J. J. (1986) Factors limiting the evidence for chemical carcinogenicity of diesel emissions in long-term inhalation experiments. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 381-396. (Developments in toxicology and environmental science: v. 13).

Waller, R. E. (1981) Trends in lung cancer in London in relation to exposure to diesel fumes. Environ. Int. 5: 479-483.

Williams, R. R.; Stegens, N. L.; Goldsmith, J. R. (1977) Associations of cancer site and type with occupation and industry from the Third National Cancer Survey interview. J. Natl. Cancer Inst. 59: 1147-1185.

Wojciak, J. F. (1988) Theoretical and experimental analyses of aerosol deposition in the lung: implications for human health effects [PhD thesis]. Rochester, NY: University of Rochester.

Wolff, R. K.; Griffis, L. C.; Hobbs, C. H.; McClellan, R. O. (1982) Deposition and retention of 0.1 $\mu$m $^{67}Ga_2O_3$ aggregate aerosols in rats following whole body exposures. Fundam. Appl. Toxicol. 2: 195-200.

Wolff, R. K.; Henderson, R. F.; Snipes, M. B.; Sun, J. D.; Bond, J. A.; Mitchell, C. E.; Mauderly, J. L.; McClellan, R. O. (1986) Lung retention of diesel soot and associated organic compounds. In: Ishinishi, N.; Koizumi, A.; McClellan, R.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, The Netherlands: Elsevier Science Publishers B. V.; pp. 199-211. (Developments on toxicology and environmental science: v. 13).

Wong, O.; Morgan, R. W.; Kheifets, L.; Larson, S. R.; Whorton, M. D. (1985) Mortality among members of a heavy construction equipment operators union with potential exposure to diesel exhaust emissions. Br. J. Ind. Med. 42: 435-448.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garshick, E.; Speizer, F. E. (1988a) Estimation of the diesel exhaust exposures of railroad workers: I. current exposures. Am. J. Ind. Med. 13: 381-394.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garschick, E.; Speizer, F. E. (1988b) Estimation of the diesel exhaust exposures of railroad workers: II. national and historical exposures. Am. J. Ind. Med. 13: 395-404.

Yu, C. P.; Morrow, P. E.; Chan, T. L.; Strom, K. A.; Yoon, K. J. (1987) A nonlinear model of alveolar clearance of insoluble particles from the lung. Inhalation Toxicol. (premier issue): 97-107.

Yu, C. P.; Yoon, K. J. (1988) Determination of lung doses of diesel exhaust perticulates. Presented at: 5th annual conference of Health Effects Institute; Colorado Springs, CO.

Yu, C. P.; Yoon, K. J.; Chen, Y. K. (1991) Retention modeling of diesel exhaust particles in rats and humans. J. Aerosol Med. 4: 79-115.

# 12. HEALTH RISK CHARACTERIZATION FOR DIESEL ENGINE EMISSIONS

## 12.1 INTRODUCTION

The purpose of a health risk characterization is to communicate to an audience the nature and extent of possible human health hazards associated with toxic agents, the circumstances under which the hazards may be present and the confidence or uncertainty about the hazards. A risk characterization consists of several elements. The first is a determination of how likely humans are to be affected and if so, how serious are the effects. The second element is a dose-response assessment in which the onset and degree of adverse effect is correlated with exposure, and if necessary scaled to a human. The third element is an estimate of human exposure levels. This is often obtained by a combination of both actual measurements and exposure modeling. The fourth element is an integration of the dose-response data with exposure estimates to describe possible impacts on an exposed population. The final element is an analysis of the uncertainties of both the evaluation and the melding of the information into a story that can be understood by the target audience.

The characterization of health hazard potential for diesel exhaust is less straight-forward than for a single toxic agent because it is a complex mixture made up of carbon particles with numerous adsorbed compounds and a vapor phase also consisting of a variety of organic as well as inorganic components. The characterization of human exposure to diesel emissions is crude and incomplete because of the difficulty in estimating ambient exposure levels. Because, in most cases, it is impossible to separate diesel particles from others present in ambient air, exposure estimates must usually be determined indirectly.
A combining of the health hazard data and the exposure information for purposes of describing the likelihood and possible magnitude of adverse effects includes uncertainties associated with both risk and exposure estimates.

## 12.2 ACUTE EXPOSURE HAZARDS

### 12.2.1 Hazard Identification

The effects reported following short-term exposures of experimental animals were, in most cases, limited to the lungs. Pulmonary edema may occur during the first days of exposure. Aggregations of particle laden macrophages in the alveolar regions of the lungs, Type II cell proliferation and thickening of alveolar walls were also common features after several days exposure. In some cases, weight losses were reported and minimal changes in lung function were seen.

The primary effects noted in occupationally exposed individuals were symptoms of mucous membrane irritation, headache, lightheadedness, and dizziness. Diesel exhaust odor is considered unpleasant enough to induce psychological effects at high concentrations. Except for decreased expiratory flow rates over the course of a workshift in one study, few changes in pulmonary function were noted.

### 12.2.2 Dose Response for Acute Toxicity

Because pulmonary effects were seen only in experimental animals at concentrations of several milligrams per cubic meter, they are unlikely to be critical endpoints for acutely exposed humans. The probable critical effects in humans (odor, headache, and mucous membrane irritation) were shown to have thresholds for diesel exhaust diluted as much as 475-fold with clean air (i.e., about 200 $\mu g/m^3$ particle concentration). Although a no-observable-adverse-effect (NOAEL) for acute exposure was not formally determined, it appears that concentrations greater than 200 $\mu g/m^3$ may be noxious. For sensitive individuals, even lower concentrations may induce adverse effects.

## 12.3 CHRONIC NONCARCINOGENIC EXPOSURE HAZARDS

### 12.3.1 Hazard Identification

The ability of diesel exhaust to induce adverse human health effects other than cancer was assessed by evaluating both subchronic and chronic animal bioassays as well as human epidemiological data. Several epidemiological studies are available to aid in evaluating the effects of chronic exposure on occupationally exposed workers. These epidemiology studies

show an effect on pulmonary function, but are not definitive for diesel exposure, because of possible coexposure to other pollutants.

An extensive animal database is available including studies with rats, mice, hamsters, cats, and monkeys. The species with the most extensive database and the one responding at the lowest doses is the rat. The studies are consistent among all species in that the critical target site is the deep lung. In these studies, the common endpoints seen were aggregations of particle laden macrophages in the alveolar regions. This was accompanied by focal thickening of the alveolar walls, replacement of Type I alveolar cells by Type II cells, and fibrosis. These changes were most evident adjacent to the aggregations of macrophages. The severity of these inflammatory responses were directly related to exposure levels.

## 12.3.2 Dose Response for Chronic Toxicity

### 12.3.2.1 Selection of Dose-Response Data

Two studies with rats were selected for determining safe levels for chronic exposure. In these two studies, in which exposure concentrations ranged from 0.11 to 7.0 $mg/m^3$ particulate matter, a large number of endpoints were measured. The chronic animal data were consistent in identifying a threshold region, the concentration range separating the lowest concentrations at which adverse effects are observed in an experimental study, and the highest tested concentrations at which effects were not observed. Because of variations in experimental design and exposure regime, the two studies selected, as well as supporting ones examining chronic diesel emission exposures, are not directly comparable. Despite these differences, the consistency in identifying the threshold region was remarkable, adding to the confidence in the no-observed-adverse-effect level (NOAEL) that was used in derivation of the RfC.

### 12.3.2.2 Dose Measure

The dose measure is considered to be the carbon particle core. (See the discussion on cancer quantitation [Section 12.4.2.2]).

#### 12.3.2.3 Dose Equivalence Across Species

The dosimetry model described in the cancer section is used (Section 12.4.2.3). As in the cancer risk assessment, it was assumed that equivalent sensitivity occurs across species when lung dose is expressed as mass of particles per unit surface area. See Chapter 11 for a discussion of this issue.

#### 12.3.2.4 Inhalation Reference Concentration Derivation

The approach used for evaluation of the concentration-response information for diesel emissions was the derivation of an inhalation reference concentration (RfC). The RfC is defined as an estimate (with uncertainty spanning perhaps and order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risks of deleterious effects during a lifetime. Although data derived from human exposures are preferred for derivation of an RfC, the available human diesel exhaust data are inadequate due to confounding, coexposure to other particles, and inadequate exposure measurement.

After determining the no observable adverse effect level (NOAEL) for the critical effect(s), adjusting dose from animals to humans and from experimental exposure durations to continuous exposure, the modified NOAEL is divided by an uncertainty factor. The size of this factor is determined by the exposure duration, quality, and completeness of the database.

The highest NOAEL observed in the principal rat studies was 0.46 $mg/m^3$. This concentration was reduced to 0.15 $mg/m^3$ to produce an equivalent dose to the lungs of humans exposed continuously, then divided by an uncertainty factor of 30. The uncertainty factor was applied to account for variations in sensitivity among humans and between humans and rats. After these adjustments the RfC of 5 $\mu g/m^3$ was derived.

#### 12.3.2.5 Reasonableness and Utility of the Inhalation Reference Concentration

There is considerable evidence that the RfC is protective against adverse effects in human populations without large preexisting lung burdens. The critical endpoints and threshold levels agreed quite well among studies, despite differences in exposure regimes and experimental designs. Because adverse effects were noted in other organs only at

considerably higher concentrations, it is unlikely that further studies will result in lower effect levels. Furthermore, only minimal pathological effects were reported in monkeys, a species more closely related to humans, exposed to concentrations nearly fourfold greater than the NOAEL in rats.

On the other hand, for individuals with preexisting lung burdens, the inference of protection at the RfC concentration may not be correct. The possibility exists that additional particle deposition resulting from concentrations at the RfC may exceed the no-observable-adverse-level. This concern cannot be further defined at present. There is little data concerning existing particle lung burdens in the general population or the degree of adaptivity of diesel exhaust particles with other ambient particulate matter.

## 12.4 CARCINOGENIC EXPOSURE HAZARDS

### 12.4.1 Hazard Identification

As previously mentioned diesel exhaust contains a number of components that have been shown to be carcinogenic in experimental animals. Extensive studies with several strains of *Salmonella typhimurium* have unequivocally demonstrated mutagenic activity of diesel exhaust particle extracts, both with and without rat liver S9 activation. Positive results also have been reported in a variety of mammalian cell tests. Sister chromatic exchanges are increased in a variety of studies with diesel particles and diesel particle extracts. Finally, DNA abducts were increased in lung epithelial cells of rats exposed to whole diesel exhaust. Overall, the evidence for lung cell DNA damage (e.g., genotoxicity) by diesel exhaust is quite strong.

Evaluation of the mutagenic activity of diesel exhaust particles stripped of organics has not been carried out. Presently available mutagenicity assay methods may not be appropriate because particle effects are thought to be mediated indirectly through release of various factors by particle laden macrophages and other phagocytic cells. An increase in DNA abducts, however, was seen following exposure to carbon black, which is similar to the diesel particle stripped of organics.

Chronic diesel exhaust inhalation exposure has resulted in the induction of lung cancer in several long-term studies in rats and mice. Respiratory tract tumors also were induced

following instillation of either organic extracts of diesel exhaust particles, or particles "stripped" of organics, into the lungs of rats. Dermal tumors were induced by skin painting with diesel particle extracts. Overall, these studies provided a broad spectrum of positive evidence for the carcinogenicity of diesel exhaust in experimental animals. The weight-of-evidence for animal data according to EPA's 1986 Carcinogenicity Risk Assessment Guidelines is "sufficient" (Federal Register, 1986).

Increased lung cancer mortality was reported in a number of epidemiology studies. The usefulness of the epidemiology data in assessing cancer risk is reduced because of methodological limitations such as small sample size, short follow up, or lack of adequate adjustment for confounding factors. Nevertheless, some of the more recent studies, especially those of Garshick et al. (1987, 1988) were able to overcome most of these deficiencies. Collectively, the epidemiology studies show evidence of an association between inhalation of diesel exhaust and lung cancer in humans. Although the evidence for carcinogenicity in humans was in most cases positive, it is judged to be "limited" according to EPA's weight-evidence-guidelines, because the observed increases in risk were quite low and the influence of confounding factors could not be completely accounted for.

Considering both the animal and human data together, diesel exhaust has been classified as a probable human carcinogen and placed in EPA weight-of-evidence-category B1. This classification is strongly supported by positive data from short-term tests and from data regarding chemical and physical composition.

## 12.4.2 Methods for Determining Dose Response

In order to develop a quantitative risk estimate using animal bioassays, the following information is required: (1) response data from chronic bioassays of acceptable quality, (2) a suitable measure of dose, (3) a deposition and retention model for estimating dose equivalence between animals and humans, and (4) availability of a suitable high to low dose extrapolation model.

### 12.4.2.1 Selection of Dose Response Data

Human data are preferable for developing risk estimates. However, for reasons described in Chapter 11, use of the human data are, in this case considered to be inadequate

for this purpose. First of all, the relative risk ratios for the human epidemiology studies are generally only slightly greater than one. Small errors in the adjustment for possible confounding factors, especially smoking, could result in a large percentage change in the relative risk. Secondly, most exposure estimates are indirect because it is virtually impossible to separate diesel particulate matter from other particles present in the ambient environment or the workplace. Finally, although considerable efforts have been made to estimate diesel exhaust exposure for occupationally exposed groups, most of these measurements are quite recent. Because of long latency periods for tumor development, exposure levels 20 to 30 years previously are necessary for accurate dose-response estimates. Because of improvements in engine efficiency and workplace ventilation, past exposure levels can, in most cases, only be guessed at. Finally, an attempt was made to use the Garshick et al. (1987) railroad worker study to develop a unit risk estimate. However, attempts to relate increasing duration or intensity of exposure to increasing response rates were unsuccessful.

On the other hand, several well-controlled and designed chronic exposure studies using rats have been carried out. Rats were the only animal species producing definitive carcinogenic effects. Despite some uncertainties in extrapolating results of animal studies to humans, the rat data was considered to be a preferable surrogate. The rats were generally exposed at higher levels than even occupationally exposed humans, although there was some overlap. Particle concentrations in the animal studies ranged from 0.1 to as great as 12 $mg/m^3$, whereas present exposure levels for railroad workers only range up to about 0.2 to 0.3 $mg/m^3$. Earlier exposures, however, probably exceeded 1 $mg/m^3$ for certain groups such as workers in railroad engine repair facilities.

Chronic bioassays with several species have been reported. Three studies, Brightwell et al. (1986), Ishinishi et al. (1986), and Mauderly et al. (1987), were considered to be well suited for risk estimation analysis. These three studies were all of 2 years duration or longer, all used Fischer 344 rats, they collectively included about a 50-fold range of exposures and all showed lung tumor induction.

#### 12.4.2.2 Dose Measure

Three measures of dose were considered: (1) the vapor phase, (2) the particle adsorbed compounds (i.e., extractable organics), and (3) the carbon core of the particle. The vapor

phase was not selected for two reasons. First of all, increases in lung cancer incidence in animals were not reported following exposure to the vapor phase alone. Although the possibility of positive responses at higher doses cannot be ruled out, the lack of positive data precludes development of a unit risk estimate based upon this component. Secondly, although the vapor phase contains some potentially carcinogenic agents, their site of action has been other than the deep lung, the primary target for diesel effects.

The particle adsorbed organic fraction does contain compounds known to induce lung cancer in experimental animals. This fraction was considered, but not ultimately selected, because the concentration of these compounds were very low and unlikely to induce the degree of carcinogenic response seen. For example, in one study (Tong and Karasek, 1984), the total concentration of 43 PAHs measured was only about 5 $\mu$g/mg of particulate matter. Furthermore only a few of these are known to be carcinogenic. Among the carcinogenic ones, the concentration of B[*a*]P was less than 0.1 $\mu$g/mg of particulate matter. Nevertheless, possible effects of the adsorbed organics cannot be totally discounted and they are considered likely to contribute to the tumorigenic responses.

The carbon core of the particle was selected as the preferred dose measure because it appears capable of not only inducing carcinogenic effects but accounting for the total animal response seen. Diesel particles stripped of adorbed organics induced lung cancer following intratracheal instillation. Inhalation of carbon black, which is similar to the carbon core of the diesel particle, resulted in about the same degree of carcinogenic response as whole diesel exhaust.

#### 12.4.2.3 Dose Equivalence Across Species

A dosimetry model was used to account for species differences in respiration rates; particle deposition efficiency; particle clearance rates, both between species and from high to low dose; transport of particles to lung associated lymph nodes; and lung surface area. Owing to the availability of much research on dosimetry in the rat and human lung, the use of such a model was preferable to using other types of assumptions to accomplish the same purpose. The dosimetry was based upon the assumption that an equivalent particle concentration per unit of lung surface area will result in an equivalent tumorigenic response

in rats and humans. The model's parameters were largely based upon experimentally derived values rather than assumptions. Details of the model are shown in Appendix D.

Particle clearance is a particularly important issue as it influences the length of time the diesel exhaust residue resides at the target tissue site. Rates of particle clearance from the deep lung differ among species, with rats having normal clearance half-times of about 2 mo versus 1 year in humans. This will result in a larger buildup of particles in human than rat lungs. Offsetting this difference to some extent is the finding that clearance is slowed at some of the higher concentrations used in the rat bioassays.

The particle associated organics, on the other hand, are eluted sufficiently rapidly that lung burdens of this component are only marginally influenced by particle clearance rates. Vapor phase components are also relatively unaffected by lung burden. Thus, depending upon which components of diesel exhaust are considered primarily responsible for lung cancer induction, differing estimates of human target tissue dose, and, thereby, estimates of risk will be obtained.

#### 12.4.2.4 High-to-Low-Dose Risk Extrapolation

The selection of a model for low-dose extrapolation depends upon the availability of information concerning mechanisms of toxic action. The EPA's 1986 carcinogenicity guidelines (Federal Register, 1986) provide the flexibility in model selection, if available information about mechanisms is sufficient to guide model selection. In the absence of such information, the guidelines recommend a default nonthreshold curve fitting model in the observed data range that has a linear component in the low-extrapolated-dose range.

It is possible that lung cancer is primarily the consequence of cytotoxity and subsequent cell proliferation induced by secretions from particle laden macrophages. The likelihood exists, however, that particle adsorbed organics or even vapor phase compounds will induce genetic alteration as well as interact in the proliferative phase. Because of the complexities of the process, uncertainties concerning the actual mechanisms and lack of quantitative data concerning interrelationships of the components, a departure from a linearized model would be arbitrary. Unit risks were therefore calculated based on two different models, both of which are linearized in the low-dose range.

*Linearized Multistage Model*

This model is a default choice that is used if another model has not been shown to be more appropriate. The LMS cannot test hypotheses about possible mechanisms of action, it is a curve-fitting model, and its main purpose is to provide upper bound low-dose risk estimates when it can be plausibly assumed that the dose-response is operating in a linear manner below the observable response range. Both malignant as well as combined malignant plus benign tumor data were modeled, as would normally be the case.

*Alternative Biologically Based Model*

This model was developed to account for the initiating and proliferative effects of both the particle adsorbed organic matter as well as the inorganic particle core. The model has the following properties.

1. Accounts for possible effects of both the carbon particle and its associated organics.
2. Allows evaluation of the contribution of both the carbon particles and organics to tumor induction.
3. Allows for changing of parameters with increasing lung burdens.
4. Assumes that cell proliferation and tumor induction are stochastic. For instance, it is not appropriate to assume that all cells divide at the same rate.

This model, which is illustrated in detail in Appendix C, has the flexibility to consider the role of the initiating properties of both the particulate matter and the organic fraction as well as possible proliferative effects of the carbon fraction. The possible proliferative effects of organics is not covered in this model because the inert particles alone seem to be able to produce a response similar to whole diesel exhaust, whereas the low concentration of toxic organics is unlikely to significantly increase cell death and proliferation.

Most of the data needed to fit the model parameters are lacking, though some can be approximated by curve fitting the model to the observed tumor incidence and mortality data in the experimental exposure range for the rat. This results in an increased uncertainty of risk estimates because of the lack of data at low doses. The model, nevertheless, provides an opportunity to test hypotheses about the relative role of initiation and proliferation and

organics versus particles in risk estimation. The model in its present form also provides an upper bound estimate of risk.

## 12.4.3 Results of Dose-Response Calculations

### 12.4.3.1 Results Using the Linearized Multistage Model

The results are summarized in Table 12-1. The LMS model was applied to three rat bioassay data sets (Brightwell et al., 1986; Ishinishi et al., Mauderly et al., 1987). The unit risk estimates calculated from these three studies varied from 1.6 to 7.1 $\times$ $10^{-5}(\mu g/m^3)^{-1}$ with a geometric mean for the three studies of 3.4 $\times$ $10^{-5}(\mu g/m^3)^{-1}$. The unit risks are the estimated 95% upper confidence limits of the risk from continuous lifetime exposure to 1 $\mu g/m^3$ of diesel exhaust particulate matter. The upper 95% confidence limit means that the true risk, which cannot be defined, may be less but is unlikely to be more than the calculated value.

**TABLE 12-1. UNIT RISK ESTIMATES PER MICROGRAMS PER CUBIC METER OF DIESEL EXHAUST**

| Reference | Model Used | 95% Upper Bound of Risk |
|---|---|---|
| Mauderly et al. (1987) | LMS | $3.4 \times 10^{-5}$ |
| Ishinishi et al. (1986) | LMS | $1.6 \times 10^{-5}$ |
| Brightwell et al. (1986) | LMS | $7.1 \times 10^{-5}$ |
| Geometric mean of above | LMS | $3.4 \times 10^{-5}$ |
| Mauderly et al. (1987) | LMS[a] | $1.7 \times 10^{-5}$ |
| Mauderly et al. (1987) | AM[a] | $1.7 \times 10^{-5}$ |
| Mauderly et al. (1987) | AM[a] | $8.2 \times 10^{-6}$ [b] |

[a]Using malignant tumors only.
[b]Maximum likelihood estimate of risk.
LMS = Linearized multistage.
AM = Alternative model.
MLE = Maximum likelihood estimate.

#### 12.4.3.2 Results Using the Alternative Model

The data from the Mauderly et al. (1987) study were applied to the alternative model. These data are useful because they contain information on natural mortality and serial sacrifice of animals, valuable for estimating tumor latency. The model parameters were based upon malignant tumors, rather than all tumors as was done in the LMS method. This was necessary in order to utilize mortality data. In order to compare the results with the LMS model, a unit risk was calculated using the LMS model applied to only the malignant tumor data from the Mauderly et al. (1987) study. The results were identical, $1.7 \times 10^{-5}$ $(\mu g/m^3)^{-1}$, when rounded. This was not unexpected, however, because curve fitting and linearization may be controlling results more than the model form. The maximum likelihood estimate (MLE) was $8.2 \times 10^{-6}$ $(\mu g/m^3)^{-1}$.

The effects of varying model coefficients are shown in Appendix C. The model shows that tumor response is very sensitive to an increase in proliferation rate of the initiated cell population and that a small change in proliferation rate will likely cause a disproportionately large change in tumorigenicity and thus risk. Results from the model also suggest that if neither the organics nor particles have an initiating effect, then the resulting risk is significantly less compared to a case where both initiation and proliferation are actively induced by the organic and particle fractions.

### 12.4.4 Discussion of Confidence in the Upper Bound Risk Estimates

The risk estimates derived are based upon well-designed, well-conducted, and adequately reported rat studies, covering a wide range of doses. The use of a species-specific dosimetry model allows a more accurate estimation of equivalent lung burdens. Finally, epidemiology studies indicate that humans are susceptible to diesel exhaust-induced cancer and that the primary target organ is the same in both rats and humans. Similar target sites increase the chance that cancer potency will not differ greatly between species.

Nevertheless, a variety of uncertainties are present, in the estimation of risk from animal data, even at doses in the observable range. Several of these involve extrapolation across species and are common to most risk assessments involving use of animal data. Even though the dosimetry model can provide a plausible, if not reasonable dose extrapolation across species, the possibility of species differences in target site sensitivity exists. In some

cases, this can be reduced by the availability of information regarding metabolic pathways, activation rates, etc. Such data are quite limited for diesel exhaust because of the complexity of the mixture. Human epidemiology data, nevertheless, suggest, at least qualitatively, that both the rat and human are more comparable in sensitivity than grossly different to diesel exhaust-induced tumors.

The unit risk estimates for both the LMS and alternative model were derived using concentration of diesel particulate matter per unit lung surface area as the dosimeter. Although this means of estimating dose is not commonly used in EPA risk assessments, it was considered to be the most plausible assumption because the diesel exhaust-induced lung tumors have been reported to arise from epithelial tissue lining the lung. Although such a dosimetric adjustment was considered to be the most reasonable, because of species differences in total numbers of lung cells, along with the possibility that some tumors may arise from other cells, a degree of uncertainty still remains regarding adoption of this parameter.

Unlike most previous assessments, an adjustment for species differences in metabolic rate was not made. Such an adjustment is based on the assumption that because of slower rates of metabolism with accompanying slower rates of detoxification, and/or repair mechanisms the effective dose in humans is less, per unit body weight, than in the smaller mammal. In the case of particles, however, it is believed that the primary cause of cancer induction is not a direct effect of xenobiotics upon lung epithelial cells. Rather, it is the result of various mediators secreted by macrophages, which diffuse to the epithelial cells, following ingestion of particles. *The greater predicted sensitivity of human lung epithelial cells, due to lower rates of detoxification, is likely to be offset by slower production of these transforming agents.* Such an assumption, although reasonable, is yet unproven.

Possible errors in selecting the fraction of exhaust used as the dosimeter also adds some uncertainty to the unit risk estimates. The organic fraction cannot be totally dismissed from playing a role since it does include a variety of carcinogenic compounds. Substituting particle adsorbed organics into the dosimetry model, however, would result in a less than twofold change in the unit risk estimates. Moreover, in an earlier EPA-sponsored effort, a unit risk estimate using in vitro and mouse skin tumor data from particle extracts and based upon the comparative potency method was nearly identical to the present one

(see Table 11-1). Although the agreement may be fortuitous, nevertheless, it indicates that selection of particles as the dosimeter is not likely to be a large source of error.

A prime area of uncertainty is the shape of the dose-response curve. For many organic agents, it is sometimes reasonable to assume a straight line extrapolation to very low doses. In the case of particles, cell proliferation may be related to particle overload and the consequent secretion of cancer-inducing mediators by macrophages. This raises the possibility of a change of shape in the dose-response curve at low doses. The relationship between macrophage particle burden and activation, however, is quite complex. The secretion of various mediators by the individual macrophages may be (1) proportional to particle load; (2) disproportional, but with no threshold; or (3) without a threshold. The particles, moreover are unevenly distributed among macrophages; thus, some macrophages may be overloaded even at low doses. Finally, it is not known if the target organ response to the mediators is linear or nonlinear, until additional data show otherwise (Section 12.6.2).

Limited positive cancer data from epidemiology studies do provide some confidence for extrapolation to concentrations at least as low as those that can occur during occupational exposure. In one such study, diesel particle concentrations were about an order of magnitude lower than those resulting in detectable carcinogenicity in the animal studies. However, these concentrations are still about an order of magnitude or more greater than found in heavily travelled urban corridors.

Both the LMS and the alternative model may misrepresent risk in individuals with preexisting lung burdens of particles. Assuming that the primary cause of lung cancer is related to lung particle burdens, then the presence of all particulate matter in the lungs would have a cumulative effect upon clearance, although not all particles would be expected to be equivalent in effects upon lung clearance or toxicity. At least some particles are present in the lungs of all individuals. Certain subgroups (e.g., smokers and individuals occupationally exposed to dusty environments) may have existing lung burdens that approach those in the experimental animal studies. If lung burdens are sufficient to inhibit particle clearance, the dose-response curve for diesel carcinogenicity in such individuals may show upward curvelinearity and the risk estimation discussed in this report may even be an underestimate of risk for such individuals. The resolution of such issues is a research need.

In summary, upper bound unit risk estimates were derived using chronic bioassay data from Fischer 344 rats. Equivalent rat/human lung burdens were estimated using a dosimetry model accounting for respiration rates, deposition efficiency, particle clearance rates, and transport to lymph nodes. Dose was based upon concentration of diesel particles per unit of lung surface area. Low-dose extrapolation was carried out using either a LMS model or an alternative low-dose extrapolation model that describes the possible contribution of both initiation and cell proliferation in the cancer risk estimation. Confirming information about mechanism of action at low doses is not available, and, thus, neither modeling approach has any overriding merit in terms of providing a better estimate of risk.

The unit risk estimates from the two extrapolation models are upper bound values, each having large uncertainties as to their proximity to the true risk. Although the values are nearly identical for one rat study, this only means that within the realm of upper bound estimating there is relative agreement. Confidence in the upper bound estimates is reasonably good in the observable range, but is much less in the extrapolated range because of the complex biological issues involved. Based upon animal data, it appears that risk may decrease more rapidly than dose at low concentrations. On the other hand, lung cancer is apparently induced in humans by exposure to diesel exhaust at concentrations several-fold less (but still greater than ambient levels) than effective doses in the animal experiments. If true this may reflect greater sensitivity due to existing lung burdens of other particulate matter, confounding factors, or other factors including greater sensitivity to diesel exhaust.

## 12.5 EXPOSURE ESTIMATES

### 12.5.1 Methodology

The most recent estimates of annual average concentrations to diesel exhaust particulate matter (DPM) were published in Chapter 9 of EPA's Motor Vehicle-Related Air Toxics Study (U.S. Environmental Protection Agency, 1993). It is necessary to appreciate that diesel emissions per se are not easily distinguishable from other suspended particulate matter present in ambient air. Thus, the focus of estimating or measuring diesel exposure is directly relatable to the measurement of airborne particulate matter. The U.S. Environmental Protection Agency (1993) study used two approaches to generate exposure estimates. In the

first one (DPM), national fleet average emission factors for 1988 were multiplied by the urban and rural grams-per-mile and micrograms-per-cubic-meter conversion factors obtained from EPA's hazardous air pollution exposure model (HAP-EM:1988). Based on this approach, urban annual average concentrations were estimated to be 2 and 1.2 $\mu g/m^3$ during 1990 and 1995. Rural exposure estimates for these two dates were 1.1 and 0.6 $\mu g/m^3$, whereas nationwide estimates for these two dates were 1.8 and 1.1 $\mu g/m^3$, respectively. The nationwide estimate is a population-weighted average between rural and urban levels.

In the second method, using ambient monitoring data, total suspended particulate matter (TSP) for 1990 was determined to equal 48 $\mu g/m^3$. This can be multiplied by the percent contribution of diesel particulate matter to TSP that is estimated to be 5.12% as derived from the ratio of $3.5 \times 10^5$ metric tons/year diesel emissions to TSP of $7.5 \times 10^6$ metric tons/year. Five percent of 48 $\mu g/m^3$ is equal to 2.2 $\mu g/m^3$. Adjustments were then made for times spent in various microenvironments including indoors. This resulted in an integrated exposure estimate of 1.5 $\mu g/m^3$. The two methods therefore produce approximately comparable results.

### 12.5.2 Confidence in Exposure Estimates

The average DPM concentrations are estimated using models and require many assumptions to evaluate uncertain input variables. Calculating an upper confidence limit on the average concentration is therefore difficult. The U.S. Environmental Protection Agency (1993) study estimates have no companion numerical estimate of uncertainties. Qualitatively, one can identify some of the factors that contribute to the uncertainty of the estimates. The model used for estimating annual average exposure is based on carbon monoxide (CO) as a surrogate for DPM and motor vehicle emissions in general. In fact, almost all estimates of ambient DPM are indirect due to difficulties in separating DPM from TSP. Another limitation is that the fixed site CO monitoring data used in the model were not adjusted to account for non-motor vehicle sources of CO, because motor vehicles are thought to be the predominant source of CO in urban areas. This assumption may lead to overestimates of exposure to diesel exhaust particulate matter. On the other hand, the population classification scheme in the model was not intended to account for groups of people who are

both highly exposed and few in number (e.g., toll booth attendants). This may result in an underestimate of exposure for some of the highest exposure groups.

Exposure estimates based upon the fraction of diesel particulate matter in TSP have uncertainties relating output of both diesel exhaust as well as TSP. The diesel-related uncertainties relate to the total number of vehicle miles travelled (VMT), the relative number of VMT in each category of vehicles (automobiles, light duty trucks, etc.), and estimates of particle emission rates. For the years 1988 through 1991, emission rates were based upon adherence to emission standards. Out-of tune or faulty engines may produce considerably more particulate matter than predicted by test results or standards. Also, certification test results are not quantitatively extrapolable to the variety of vehicular driving and fuel conditions.

There is limited information available regarding highly exposed populations. It is known that they exist, but data specific to their exposure is quite limited. The Motor Vehicles Manufacturing Association carried out a detailed analysis of air pollution from diesel engine emissions in the City of Los Angeles (Sienicki and Mago, 1992). They derived a mean estimated concentration of DPM for 1995 of 2.7 $\mu g/m^3$. This is somewhat less than an earlier estimate of 4.4 $\mu g/m^3$ (U.S. Environmental Protection Agency, 1983). Differences were primarily due to lower estimates of particle emission rates by Sienicki and Mago.

McClellan (1986) estimated that workers on urban freeways and individuals in urban street canyons may be exposed to 15 $\mu g/m^3$ of DPM. The accuracy of this estimate, however, is uncertain because it was based upon data from the early 1980s when the mix of vehicles, vehicle miles travelled, and emission standards were different from the present. There was also no estimate of the number of individuals exposed at these higher concentrations.

For convenience, Table 12-2 lists the previously discussed estimates. The estimates vary depending upon what assumptions were made, and it is not clear which are better and for what reason.

**TABLE 12-2. ESTIMATED ANNUAL AMBIENT CONCENTRATIONS OF DIESEL EXHAUST PARTICULATE MATTER[a]**

| Year | U.S. Environmental Protection Agency (1993) Method 1 Rural | U.S. Environmental Protection Agency (1993) Method 1 Urban | U.S. Environmental Protection Agency (1993) Method 1 Nationwide | U.S. Environmental Protection Agency (1993) Method 2 | MVMA (1992) Los Angeles | McClellan (1986) Highly Exposed |
|---|---|---|---|---|---|---|
| 1986 | — | — | — | — | — | 15.0 |
| 1990 | 1.1 | 2.0 | 1.8 | 1.5 | — | — |
| 1995 | 0.6 | 1.2 | 1.1 | — | 2.7 | — |

[a]Expressed in micrograms per cubic meter.

## 12.6 POPULATION RISKS AND UNCERTAINTIES

With a clear understanding of hazard identification, dose-response evaluation, exposure, and the attendant uncertainties, one may choose to demonstrate the magnitude of possible health impacts upon a population by developing estimates of possible disease occurrence. For carcinogenesis this traditionally is done by combining the cancer unit risk values with the population exposure. An upper bound estimate of individual risk is thus obtained, and if population figures are available, the risk can be converted to a hypothetical number of cancer cases. In the case of noncancer toxicity, estimates of safe concentrations for either acute or chronic exposure are compared with the range of estimated exposure levels to determine the degree of exposure above safe levels, the duration of exposure above safe levels, or both.

### 12.6.1 Population Risks for the Induction of Noncancer Toxicity

#### 12.6.1.1 Population Risks for Acute Exposure

Population risks for acute exposure have not been estimated.

#### 12.6.1.2 Population Risks for Chronic Exposure

An RfC of 5 $\mu g/m^3$ is recommended based upon a NOAEL of 0.46 $mg/m^3$ in rats (adjusted to 0.15 $mg/m^3$ equivalent concentration in humans), combined with an uncertainty factor of 30. The RfC is greater than concentrations estimated to occur under most ambient conditions. In certain locations, however, such as tunnels, crowded freeways, city street canyons, etc., the concentrations of diesel exhaust particulate matter may exceed the RfC

during at least some portions of the day. However, the RfC is based on continuous lifetime exposure, and therefore high "parttime" exposure corridors need to be reevaluated in terms of long-term average exposures. See Figure 12-1 relating risk to exposure for an overall perspective.

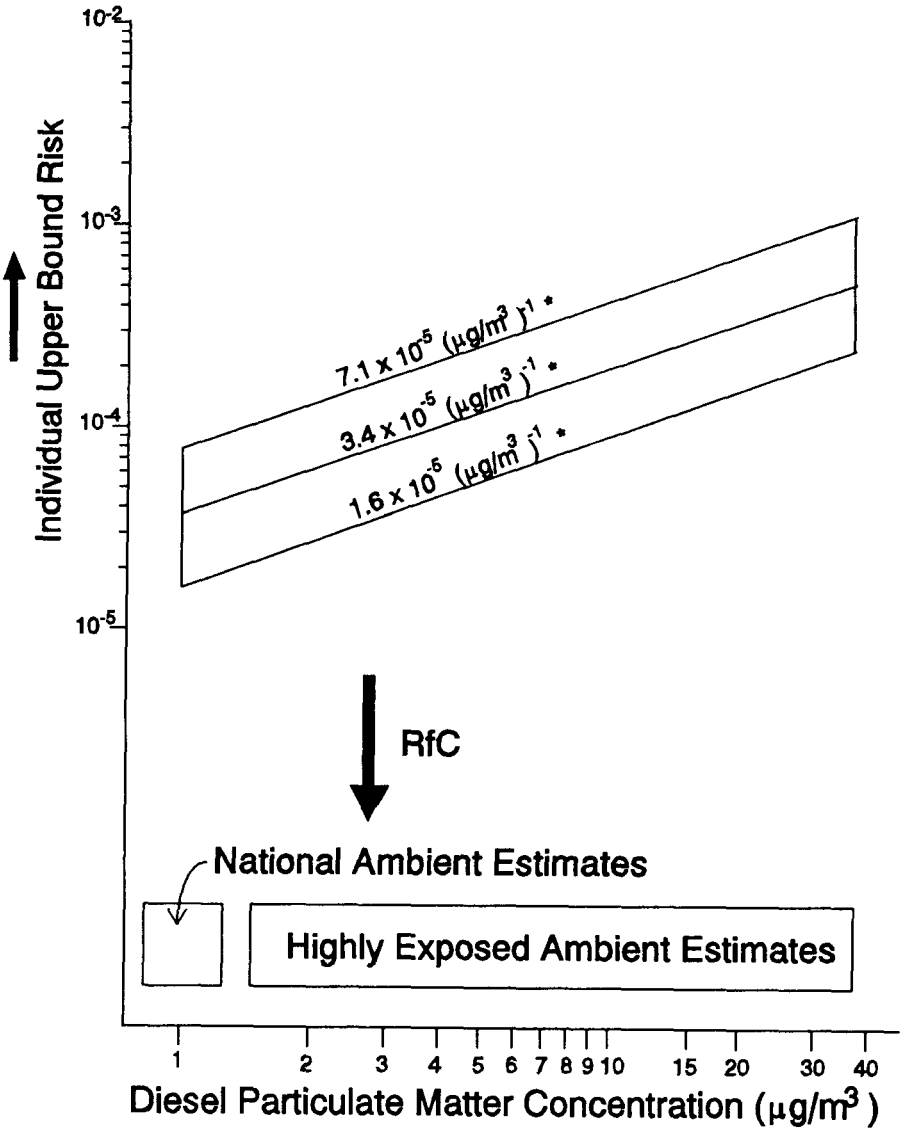


**Figure 12-1. Relationship of exposure estimates and risk-specific doses.**

### 12.6.2 Population Risks for Induction of Cancer

With a clear understanding of hazard identification, dose-response evaluation, exposure, and the attendant uncertainties, one may choose to demonstrate the magnitude of possible health impacts upon a population by developing estimates of disease occurrence. This traditionally is done by combining the cancer risk values with the population exposure to obtain an estimate of individual risk, or if population figures are available, converting the risk number to an upper bound estimate of cancer cases.

The usefulness of the cancer unit risk estimates in characterizing population risks must be considered in light of some specific information that is unique to the diesel issue. Confidence is lacking that these risk estimates are protective for some subgroups. Yet for other subgroups risk may be overestimated. Fortunately, information about this is more obvious for diesel exhaust than for other agents. The true risk at low doses may be less than predicted by a linearized low-dose extrapolation model because the proliferative effects of particles is no longer apparent at some point in the low-dose range. A threshold for tumorigenic responses is unlikely, however, because of the initiating properties of chemicals present in the organic fraction of diesel exhaust.

Although, for any population, the true risk could be lower for reasons stated above, some circumstances may result in higher risks for certain subgroups. There is concern for individuals with existing lung particle burdens (i.e., smokers and those employed in a number of occupations). Because such individuals would be expected to have a slowed particle clearance rate if lung burdens exceed a threshold, this would infer a steepening of the dose-response curve and a higher risk for diesel exhaust exposure. Greater exposure will occur in certain other subgroups such as laborers or endurance athletes due to increased respiration rates. A variety of metabolic factors (i.e., impaired ability to repair DNA) may exist in some individuals. For comparative purposes these risk estimates share some consistency of approach with many other chemicals. An improved dose-response-based risk characterization, however, cannot be pursued until a better understanding of mechanisms is achieved and data gaps are addressed.

Estimates of population exposures are available but their accuracy in representing true exposure likewise cannot be described at this time. Because of uncertainties regarding both ambient exposure levels, as well as those relating to low-dose extrapolation, outright

calculations of possible cancer cases or risk levels may be misleading unless one is aware of these uncertainties. Given these cautions, upper bound estimates of possible cancer mortality could be derived that vary from about $2 \times 10^{-5}$ for rural residents during 1995 to $7 \times 10^{-5}$ for residents of urban areas during 1990. These values are based upon the U.S. Environmental Protection Agency (1993) study average exposure estimates and the geometric mean of unit risk estimates obtained by applying the LMS model to three animal studies (see Table 12.1). Using the McClellan (1986) exposure estimate for urban street canyons of 15 $\mu g/m^3$ as a highly exposed population, an individual's risk may be as great as $5 \times 10^{-4}$. It should be emphasized that these numbers, when viewed together, provide some plausible boundaries for cancer or risk. The uncertainties of these risk estimates are qualitatively identifiable because they include uncertainties related to the unit risk estimate as well as exposure levels. For reasons discussed, risk may be overestimated under most ambient conditions for an average person. On the other hand, the actual risk may be greater for individuals performing heavy labor, exercising in polluted areas, occupationally exposed, or with lungs already burdened by large numbers of particles (see Figure 12-1).

### 12.6.3 Comparison of Cancer and Noncancer Risk Estimates

The relationship between an RfC and a unit risk is not straightfoward. Although the unit risk estimates a finite cancer rate at any exposure concentration, the RfC is a concentration assumed to be without adverse effects even among sensitive segments of the population. At the RfC concentration, an estimated cancer risk of 1.7/10,000 still exists if the assumption is made that all particle and organic fraction mechanisms are operating. Thus concentrations lower than the RfC are necessary to reduce the estimated cancer risk to less than $10^{-4}$. However, because no adverse effects, including cell proliferation, are assumed to occur at this concentration, the cancer rate may be less than $10^{-4}$ in a population without preexisting lung burdens. On the other hand, for individuals with preexisting lung particle burdens near the threshold for inhibition of clearance, the additional cancer risk may be greater than predicted.

## 12.7 SUMMARY

Diesel exhaust consists of a vapor phase and particulate matter component made up of an insoluble carbon core with sulfates and a large variety of organic compounds adsorbed to the surface. Although a number of the particle-associated and vapor phase compounds are known toxins and are likely to contribute to the disease process, the particle fraction is hypothesized to be the most influential in disease causation. The role of particle extractable organics, however, is not ruled out and probably does have some influence on the total response.

Animal studies indicate that chronic exposure to diesel exhaust can result in lung cancer, pulmonary pathology, and possibly neurotoxic effects. The primary effects noted acutely are lung damage at high-exposure concentrations.

Although human epidemiology studies indicate that diesel exhaust is likely to be carcinogenic, confounding factors cannot be completely ruled out. Diesel exhaust has been classified into cancer weight-of-evidence category B1 and, thus, is considered to be a probable human carcinogen. Noncancer effects were generally limited to minor changes in pulmonary function. Acute exposure resulted in eye irritation and headaches.

An upper bound cancer unit risk estimate of $3.4 \times 10^{-5}(\mu g/m^3)^{-1}$ is recommended. This is the upper 95% confidence limit of cancer risk from continuous lifetime exposure to 1 $\mu g/m^3$ particulate matter. There is considerable uncertainty regarding this estimate, primarily because of possible species differences in sensitivity and possible variations in slope of the dose-response curve at low exposure concentrations.

An RfC of 5 $\mu g/m^3$ is recommended. This is a concentration considered unlikely to induce noncancer toxic effects even following continuous lifetime exposure. For the majority of the population there is considerable confidence in this RfC because of general agreement in thresholds from several different animal studies and because of limited responses in occupationally exposed humans. For individuals with existing lung particle burdens near the threshold for inhibition of clearance or induction of toxic effects, however, the RfC may not be protective.

Human exposures are estimated to vary from about 1 $\mu g/m^3$ in rural areas up to about 15 $\mu g/m^3$ in heavily travelled urban streets. Although these are considered reasonable

estimates of mean exposure levels, periodic exposure to much higher, uncertain levels may occur in some portion of the population from time to time.

Except for situations such as dwellers near heavily travelled city street canyons, long-term exposures are unlikely to exceed the RfC. The effects of periodic exposure above the RfC, are uncertain although data from occupationally exposed populations suggests any toxic responses are likely to be mild and transient.

Based upon the recommended unit risk estimate and an estimate for lifetime exposure of 15 $\mu g/m^3$, the risk to humans could be as high as $5 \times 10^{-4}$. Because these are upper bounds for both the risk estimate and exposure, the actual risk is likely to be much less for healthy individuals. For individuals either with existing lung particle burdens or exposed to high concentrations of diesel exhaust, risk may be higher. It should be emphasized that these estimates are most valuable for range finding than for estimates of possible disease occurrence.

# REFERENCES


Brightwell, J.; Fouillet, X.; Cassano-Zoppi, A.-L.; Gatz, R.; Duchosal, F. (1986) Neoplastic and functional changes in rodents after chronic inhalation of engine exhaust emissions. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 471-485. (Developments in toxicology and environmental science: v. 13).

Federal Register. (1986) Guidelines for carcinogen risk assessment. F. R. (September 24) 51: 33992-34003.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1987) A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 135: 1242-1248.

Garshick, E.; Schenker, M. B.; Munoz, A.; Segal, M.; Smith, T. J.; Woskie, S. R.; Hammond, S. K.; Speizer, F. E. (1988) A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. Am. Rev. Respir. Dis. 137: 820-825.

Ishinishi, N.; Kuwabara, N.; Nagase, S.; Suzuki, T.; Ishiwata, S.; Kohno, T. (1986) Long-term inhalation studies on effects of exhaust from heavy and light duty diesel engines on F344 rats. In: Ishinishi, N.; Koizumi, A.; McClellan, R. O.; Stöber, W., eds. Carcinogenic and mutagenic effects of diesel engine exhaust: proceedings of the international satellite symposium on toxicological effects of emissions from diesel engines; July; Tsukuba Science City, Japan. Amsterdam, Holland: Elsevier Science Publishers B. V.; pp. 329-348. (Developments in toxicology and environmental science: v. 13).

Mauderly, J. L.; Jones, R. K.; Griffith, W. C.; Henderson, R. F.; McClellan, R. O. (1987) Diesel exhaust is a pulmonary carcinogen in rats exposed chronically by inhalation. Fundam. Appl. Toxicol. 9: 208-221.

McClellan, R. O. (1986) Health effects of diesel exhaust: a case study in risk assessment. Am. Ind. Hyg. Assoc. J. 47: 1-13.

Sienicki, E. J.; Mago, R. S. (1992) Re-evaluation of diesel particulate emission inventories. In: Toxic air pollutants from mobile sources: proceedings of an international specialty conference. Pittsburgh, PA: Air and Waste Management Association; pp. 151-164.

Tong, H. Y.; Karasek, F. W. (1984) Quantitation of polycyclic aromatic hydrocarbons in diesel exhaust particulate matter by high-performance liquid chromatography fractionation and high-resolution gas chromatography. Anal. Chem. 56: 2129-2134.

U.S. Environmental Protection Agency. (1983) Diesel particulate study. Ann Arbor, MI: Office of Mobile Sources.

U.S. Environmental Protection Agency. (1991a) National air pollutant emission estimates 1940—1990. Research Triangle Park, NC: Office of Air Quality Planning and Standards; EPA report no. EPA/450/4-91/028. Available from: NTIS, Springfield, VA; PB92-152859/XAB.

U.S. Environmental Protection Agency. (1991b) National air quality and emissions trends report, 1990. Research Triangle Park, NC: Office of Air Quality Planning and Standards; EPA report no. EPA-450/4-91-023. Available from: NTIS, Springfield, VA; PB92-141555/XAB.

U.S. Environmental Protection Agency. (1993) Motor vehicle-related air toxics study. Ann Arbor, MI: Office of Mobile Sources; EPA report no. EPA/420/R-93/005. Available from: NTIS, Springfield, VA; PB93-182590/XAB.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garshick, E.; Speizer, F. E. (1988a) Estimation of the diesel exhaust exposures of railroad workers: I. current exposures. Am. J. Ind. Med. 13: 381-394.

Woskie, S. R.; Smith, T. J.; Hammond, S. K.; Schenker, M. B.; Garschick, E.; Speizer, F. E. (1988b) Estimation of the diesel exhaust exposures of railroad workers: II. national and historical exposures. Am. J. Ind. Med. 13: 395-404.

# APPENDIX A

# EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | |
|---|---|---|---|---|---|
| Facility/Sponsor | U.S. Environmental Protection Agency | | | | |
| Reference | Bhatnager et al., 1980; Campbell et al., 1980, 1981; Hyde et al., 1985; Moorman et al., 1985; Pepelko et al., 1980b, 1981; Pepelko, 1982b; Pepelko and Peirano, 1983; Plopper et al., 1983 | | | Laurie et al., 1980; Laurie and Boyes, 1980, 1981 | |
| Engine type | Nissan CN 6-33, 3.24 L, 6-cylinder | | | 3.24 L, 6 cylinder | |
| Operating mode | Federal short cycle | | | Federal short cycle | |
| Fuel type | No. 2 diesel | | | No. 2 diesel | |
| Fuel sulfur | 0.15% | | | 0.15% | |
| Exposure regime | 8 h/d, 7 d/week, 124 weeks | | | 8 h/d, 7 d/week, 16 weeks | |
| Exposure conditions | Control | Exhaust - weeks 1-61 | Exhaust - weeks 62-124 | Control | Exhaust |
| Particle conc. (mg/m$^3$) | 0.00 | 6.34 ±0.81 | 11.70 ±0.99 | 0.01 | 5.97 ±0.17[b] |
| Particle size | | 90% < 1 µm by mass; 50% ≤ 0.3 µm by mass | | | |
| $CO_2$ (%) | 0.04 ±0.002 | 0.30 ±0.04 | 0.52 ±0.04 | 0.05 ±0.00[b] | 0.28 ±0.01[b] |
| CO (ppm) | 2.20 ±0.50 | 20.17 ±3.01 | 33.30 ±2.94 | 1.86 ±0.06[b] | 19.20 ±0.35[b] |
| $NO_2$ (ppm) | 0.03 ±0.03 | 2.68 ±0.80 | 4.37 ±1.19 | 0.03 ±0.00[b] | 2.51 ±0.10[b] |
| NO (ppm) | 0.05 ±0.04 | 11.64 ±2.34 | 19.39 ±3.80 | 0.08 ±0.01[b] | 11.14 ±0.43[b] |
| $SO_2$ (ppm) | 0.03 ±0.02 | 2.12 ±0.58 | 5.03 ±1.03 | 0.46 ±0.02[b] | 1.82 ±0.07[b] |
| $SO_4^{-2}$ (µg/m$^3$) | - | - | - | | |
| Ozone (ppm) | | | | | |
| Aliphatic aldehydes (ppm) | 0.00 | 0.177 ±0.043 | 0.338 ±0.057 | | |
| Formaldehyde (ppm) | 0.00 | 0.106 ±0.029 | 0.251 ±0.059 | | |
| Acrolein (ppm) | 0.00 | 0.025 ±0.003 | 0.034 ±0.009 | | |
| $NH_4^+$ | - | - | - | | |
| THC (ppm) | 2.82 ±0.50 | 7.93 ±1.42 | 11.02 ±1.04 | 3.22 ±0.08[b] | 7.29 ±0.11[b] |
| PAHs | | | | | |
| Benzo[a]pyrene | | 15.9 µg/g extract | | | |
| Benzo[e]pyrene | | 28.6 µg/g extract | | | |
| Benzo[a]anthracene | | 53.8 µg/g extract | | | |
| Benzo[k]fluoranthene | | 77.8 µg/g extract (k+b) | | | |
| Fluoranthene | | 155.8 µg/g extract | | | |
| Pyrene | | 198 µg/g extract | | | |
| Phenanthrene | | 145.2 µg/g extract | | | |

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | |
|---|---|---|---|---|
| Facility/Sponsor | U.S. Environmental Protection Agency | | | |
| Reference | Bhatnager et al., 1980; Campbell et al., 1980, 1981; Hyde et al., 1985; Moorman et al., 1985; Pepelko et al., 1980b, 1981; Pepelko, 1982b; Pepelko and Peirano, 1983, Plopper et al., 1983 | | Laurie et al., 1980; Laurie and Boyes, 1980, 1981 | |
| Coronene | | 71.6 µg/g extract | | |
| Perylene | | 3.5 µg/g extract | | |
| Indeno(1,2,3-Cd) fluoranthene | | 10.9 µg/g extract | | |
| Indeno(1,2,3-Cd) pyrene | | 14.8 µg/g extract | | |
| Benzo(ghi)perylene | | 21.1 µg/g extract | | |

a All ± are S.D. unless specified otherwise.

b Standard error of mean values.

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | |
|---|---|---|---|---|
| Facility/Sponsor | U.S. Environmental Protection Agency | | | |
| Reference | Wiester et al., 1980 | | | Pepelko et al., 1980a |
| Engine type | Nissan CN6-33, 3.24 L, 6 cylinder | | | 3.24 L, 6 cylinder |
| Operating mode | California cycle, modified | | | California cycle, modified |
| Fuel type | No. 2 diesel | | | No. 2 diesel |
| Fuel sulfur | 0.15% | | | |
| Exposure regime | 20 h/d, 7 d/week, 4 weeks | | | 20 h/d, 7 d/week, 4 weeks |
| Exposure conditions | Control | Exhaust | Exhaust - irradiated | Exhaust |
| Particle conc. ($mg/m^3$) | 0.00 | 6.32 ±1.31 | 6.83 ±1.44 | 6.40 ±0.36[b] |
| Particle size | | 0.1-1.0 μm | | |
| $CO_2$ (%) | 0.04 | 0.261 ±0.01 | 0.25 ±0.03 | 0.26 ±0.008[b] |
| CO (ppm) | 2.0 | 17.4 ±2.5 | 16.7 ±4.0 | 14.61 ±0.90[b] |
| $NO_2$ (ppm) | 0.07 | 2.3 ±0.4 | 2.9 ±0.7 | 2.13 ±0.09[b] |
| NO (ppm) | 0.11 | 5.9 ±0.6 | 5.0 ±1.2 | 6.13 ±0.18[b] |
| $SO_2$ (ppm) | 0.0 | 2.1 ±0.8 | 1.9 ±0.8 | 2.10 ±0.21[b] |
| $SO_4^{-2}$ ($\mu g/m^3$) | 0.00 | 0.57 ±0.12 | 0.57 ±0.13 | 0.577 ±0.019[b] |
| Ozone (ppm) | 0.0 | 0.0 | <0.01 | |
| Aliphatic aldehydes (ppm) | | | | |
| Formaldehyde (ppm) | | | | |
| Acrolein (ppm) | | | | |
| $NH_4^+$ | | | | |
| THC (ppm) | 0.00 | 31.6 ±2.3 | 26.1 ±1.6 | 31.56 ±1.25[b] |
| PAHs<br>Benzo[a]pyrene | | | | |
| Nitropyrene | | | | |

a All ± are S.D. unless specified otherwise.

b Standard error of mean values.

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | | |
|---|---|---|---|---|---|---|
| Facility/Sponsor | U.S. Environmental Protection Agency | | | | | |
| Reference | Pepelko, 1982a | | | Lee et al., 1978, 1980 | | |
| Engine type | Nissan, 6 cylinder, 3.24 L | | | 3.24 L, 6 cylinder | | |
| Operating mode | California cycle, modified | | | California cycle, modified | | |
| Fuel type | No. 2 diesel | | | No. 2 diesel | | |
| Fuel sulfur | | | | | | |
| Exposure regime | 20 h/d, 7 d/week, 4 weeks | | | 20 h/d, 9 weeks | | |
| Exposure conditions | Control | Exhaust | Exhaust - irradiated | Control | Exhaust | Exhaust - irradiated |
| Particle conc. ($mg/m^3$) | | 6.40 ±0.36 | 6.75 ±0.39 | | 6.32 | 6.83 |
| Particle size (μm) MMD[b] (GSD)[c] | | | | | | |
| $CO_2$ (%) | | 0.247 ±0.003 | 0.244 ±0.007 | 0.040 | 0.252 | 0.255 |
| CO (ppm) | | 16.9 ±1.1 | 16.1 ±1.3 | 2.0 | 15.7 | 15.4 |
| $NO_2$ (ppm) | | 2.49 ±0.18 | 2.76 ±0.15 | 0.07 | 2.19 | 2.73 |
| NO (ppm) | | 5.71 ±0.21 | 4.53 ±0.15 | 0.11 | 5.85 | 4.94 |
| $NO_x$ (ppm) | | | | | | |
| $SO_2$ (ppm) | | 2.10 ±0.21 | 1.86 ±0.21 | | 2.13 | 1.91 |
| $SO_4^{-2}$ ($\mu g/m^3$) | | 577 ±19 | 569 ±19 | 0.0 | 0.57 | 0.57 |
| $O_2$ (%) | | | | | | |
| Ozone (ppm) | | | | | | <0.01 |
| Aliphatic aldehydes | | | | | | |
| Formaldehyde (ppm) | | | | | | |
| Acrolein (ppm) | | | | | | |
| $NH_4^+$ | | | | | | |
| Hydrocarbons (ppm) | | 31.6 ±3.8 | 26.1 ±3.4 | 2.0 | 15.6 | 15.0 |
| PAHs<br>Benzo(a)pyrene | | | | | | |
| Nitropyrene | | | | | | |

[a] All ± are standard errors of weekly means.
[b] Mass median diameter
[c] Geometric standard deviation.

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| | | | | |
|---|---|---|---|---|
| Facility/Sponsor | National Institute for Occupational Safety and Health | | | |
| Reference | Castranova et al., 1985; Fedan et al., 1985; Habon et al., 1985; Lewis et al., 1986, 1989; Mentnech et al., 1984; Vallyathan et al., 1986 | | | |
| Engine type | Caterpillar 3304, 7 L, 4-cycle | | | |
| Operating mode | 8-mode mining cycle, 60% idling | | | |
| Fuel type | No. 2 diesel | | | |
| Fuel sulfur | 0.34% | | | |
| Exposure regime | 7 h/d, 5 d/week, 104 weeks | | | |
| Exposure conditions | Control | Exhaust | Coal dust | Exhaust + coal dust |
| Particle conc. ($mg/m^3$) | | | 4.98 ±0.82 | 3.23 ±0.60 |
| Respirable particles[b] ($mg/m^3$) | | 1.95 ±0.25 | 2.00 ±0.41 | 2.02 ±0.30 |
| Particle size (μm) MMD[c] (GSD)[d] | | 0.23 (±2.5)[e]<br>0.36 (±2.0)[f] | | |
| $CO_2$ (%) | 0.08 ±0.02 | 0.20 ±0.06 | 0.09 ±0.05 | 0.20 ±0.07 |
| CO (ppm) | 2.2 ±0.9 | 11.5 ±3.1 | 2.2 ±0.9 | 10.9 ±2.8 |
| $NO_2$ (ppm) | 0.06 ±0.04 | 1.5 ±0.5 | 0.06 ±0.05 | 1.6 ±0.5 |
| NO (ppm) | 0.08 ±0.14 | 8.7 ±3.6 | 0.08 ±0.29 | 8.3 ±3.2 |
| $SO_2$ (ppm) | | 0.81 ±0.38 | 0.01 ±0.07 | 0.61 ±0.29 |
| $SO_4^{-2}$ ($\mu g/m^3$) | | 29.0 ±24.9 | 16.8 ±17.9 | 42.3 ±33.8 |
| Aliphat. aldehydes (ppm) | 0.02 ±0.01 | 0.12 ±0.06 | 0.02 ±0.01 | 0.12 ±0.05 |
| Formaldehyde (ppm) | 0.0076 ±0.0035 | 0.0383 ±0.0230 | 0.0074 ±0.0041 | 0.0374 ±0.0266 |
| Acetaldehyde (ppm) | 0.0015 ±0.0035 | 0.0387 ±0.0153 | 0.0009 ±0.0025 | 0.0377 ±0.014 |
| Acrolein (ppm) | 0.0030 ±0.0033 | 0.0602 ±0.0245 | 0.0062 ±0.0047 | 0.0578 ±0.0205 |
| $NH_3$ (ppm) | 0.52 ±0.28 | 0.64 ±0.71 | 0.57 ±0.52 | 0.48 ±0.55 |
| $NH_4^+$ (ppm) | | 0.027 ±0.0307 | 0.0065 ±0.0143 | 0.0165 ±0.0233 |
| THC (ppm) | 4.1 ±1.9 | 7.5 ±2.2 (cold) | | 7.4 ±2.0 (cold) |
| PAH ($\mu g/m^3$) Benzo(a)pyrene | | 13.5 ±6.8 | | 10.2 ±6.5 |
| Benzo(a)anthracene | | 19.6 ±9.9 | 3.2 ±2.2 | 11.2 ±5.2 |
| Benzo(k)fluoranthene | | 5.6 ±2.3 | | 3.6 ±2.4 |
| Fluoranthene | | 139.3 ±98.1 | 26.5 ±11.5 | 67.5 ±52.4 |
| Pyrene | | 123.4 ±72.2 | 32.3 ±15.1 | 60.0 ±36.6 |

[a] All ± are S.D., unless specified otherwise
[b] < 7μm
[c] Mass median diameter
[d] Geometric standard deviation
[e] Electrical aerosol size analyzer
[f] Scanning electron microscope.

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| Facility/Sponsor | National Institute for Occupational Safety and Health | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Reference | Green et al., 1983, Rabovsky et al., 1986 | | | | Rabovsky et al., 1984 | | | |
| Engine type | Caterpillar, 7 L, 4 cylinder, 4-cycle | | | | Caterpillar, 7 L, 4 cylinder, 4-cycle | | | |
| Operating mode | 8-mode mining cycle, 60% idling | | | | 8-mode mining cycle, 60% idling | | | |
| Fuel type | No. 2 diesel | | | | No. 2 diesel | | | |
| Fuel sulfur | < 0.5% | | | | | | | |
| Exposure regime | 7 h/d, 5 d/week, 12 mo. | | | | 7 h/d, 5 d/week, 24 mo | | | |
| Exposure conditions | Control | Exhaust | Coal dust | Exhaust + coal dust | Control | Exhaust | Coal dust | Exhaust + coal dust |
| Particle conc. ($mg/m^3$) | | 2 | 5 | 3 | | | | |
| Respirable particles[b] ($mg/m^3$) | | 2.01 | 1.97 | 2.08 | | 1.9 ±0.3 | 2.1 ±0.4 | 2.0 ±0.3 |
| Particle size (μm) MMD[c] (GSD)[d] | | | | | | | | |
| $CO_2$ (%) | 0.08 | 0.21 | 0.09 | 0.20 | 0.07 ±0.02 | 0.16 ±0.04 | 0.08 ±0.04 | 0.17 ±0.06 |
| CO (ppm) | 2.3 | 12.7 | 2.4 | 11.1 | 2.0 ±0.9 | 10.5 ±2.3 | 2.1 ±0.8 | 10.3 ±2.0 |
| $NO_2$ (ppm) | 0.04 | 1.6 | 0.04 | 1.3 | 0.06 ±0.04 | 1.5 ±0.5 | 0.07 ±0.05 | 1.5 ±0.05 |
| NO (ppm) | 0.07 | 9.7 | 0.08 | 1.3 | 0.08 ±0.13 | 7.8 ±3.1 | 0.08 ±0.28 | 7.6 ±2.8 |
| $SO_2$ (ppm) | 0.01 | 0.83 | | 0.56 | | 0.6 ±0.4 | 0.003 ±0.05 | 0.5 ±0.3 |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | | | | | |
| Aliphatic aldehydes | | | | | | | | |
| Formaldehyde (ppm) | | | | | | | | |
| Acetaldehyde (ppm) | | | | | | | | |
| Acrolein (ppm) | | | | | | | | |
| $NH_3$ (ppm) | 0.63 | 1.13 | 0.83 | 0.54 | 0.5 ±0.6 | 0.6 ±0.8 | 0.6 ±0.7 | 0.4 ±0.3 |
| $NH_4^+$ (ppm) | | | | | | | | |
| THC (ppm) | | | | | | | | |
| PAH ($\mu g/m^3$) Benzo(a)pyrene | | | | | | | | |
| Nitropyrene | | | | | | | | |

[a] All ± are S.D. unless specified otherwise.
[b] < 7μm
[c] Mass median diameter
[d] Geometric standard deviation

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | | |
|---|---|---|---|---|---|---|
| Facility/Sponsor | General Motors Research Lab | | | | | |
| Reference | Barnhart et al., 1981, 1982; Chaudhari et al., 1980, 1981; Chaudhari and Dutta, 1982; Chen and Vostal, 1981; Dziedzic, 1981; Eskelson et al., 1981; Penney et al., 1981: Misiorowski et al., 1980, 1981; Navarro et al., 1981; Schneider and Felt, 1981; Schreck et al., 1980, 1981; Strom, 1984; Vostal et al., 1981; Wallace et al., 1987; White and Garg, 1981 | | | | Gross, 1981 | |
| Engine type | 1978 350D Oldsmobile, 5.7 L, 4-cycle | | | | 5.7 L | |
| Operating mode | 1350 rpm, 96 N-m | | | | 1350 rpm, 96 N-m | |
| Fuel type | Amoco type 2D | | | | Amoco type 2D | |
| Fuel sulfur | 0.27% | | | | 0.27% | |
| Exposure regime | 20 h/d, 5½ d/week, 104 weeks | | | | 20 h/d, 5½ d/week, 87 weeks | |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust | Control | Exhaust |
| Particle conc. (mg/m$^3$) | 0.007 ±0.009 | 0.258 ±0.087 | 0.796 ±0.228 | 1.533 ±0.346 | 0.007 ±0.009 | 1.533 ±0.346 |
| Particle size (µm) MMD[b] (GSD) | | 0.19 | | | | 0.2 |
| $CO_2$ (%) | | | | | | |
| CO (mg/m$^3$) | 2.2 ±0.6 | 3.4 ±0.8 | 5.3 ±0.9 | 7.9 ±2.1 | 1.9 | 7 |
| $NO_2$ (ppm) | | | | | | 0.5 |
| NO (ppm) | | | | | | 6.7 |
| $NO_x$ (mg/m$^3$) | 0.05 | 2.1 ±0.6 | 5.0 ±1.2 | 9.2 ±1.6 | <0.04 | 7.2 |
| Sulfur (mg/m$^3$) | | | | | | 1.4 |
| $SO_2$ (ppm) | | | | | | |
| Aliphatic aldehydes | | | | | | |
| Formaldehyde (ppm) | | | | | | |
| Acrolein (ppm) | | | | | | |
| $NH_4^+$ | | | | | | |
| THC (ppm) | | | | | | |
| PAHs<br>Benzo(a)pyrene | | | | | | |
| Nitropyrene | | | | | | |

[a] All ± are S.D., unless specified otherwise.
[b] Mass median diameter.

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | |
|---|---|---|---|---|
| Facility Sponsor | Inhalation Toxicology Research Institute | | | |
| Reference | Bice et al., 1985; Cheng et al., 1984; Henderson et al., 1983, 1985, 1988; Mauderly et al., 1983, 1984, 1987a, b, 1988; McClellan et al., 1986; Wolf et al., 1987 | | | |
| Engine type | 1980 Oldsmobile V8, 5.7 L | | | |
| Operating mode | Federal Test Procedure, urban driving cycle | | | |
| Fuel type | Phillips No. 2 diesel | | | |
| Fuel sulfur | 0.34% | | | |
| Exposure regime | 7 h/d, 5 d/week, 130 weeks | | | |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust |
| Particle conc ($mg/m^3$) | 0.013 ±0.006 | 0.353 ±0.071 | 3.469 ±0.447 | 7.082 ±0.808 |
| Particle size (μm) MMD[b] (GSD)[c] | | 0.183 ±0.04 (4.8 ±0.28)[d] 0.262 ±0.06 (4.2 ±0.24)[e] | 0.184 ±0.02 (5.3 ± 0.64)[d] 0.249 ±0.03 (4.5 ±0.54)[e] | 0.213 ±0.06 (4.7 ±0.94)[d] 0.234 ±0.06 (4.4 ±0.88)[e] |
| $CO_2$ (%) | 0.2005 ±0.0390 | 0.2284 ±0.0371 | 0.4355 ±0.0590 | 0.6643 ±0.1320 |
| CO (ppm) | 1.0 ±0.7 | 2.9 ±1.0 | 16.5 ±7.1 | 29.7 ±12.9 |
| $NO_2$ (ppm) | 0 | 0.05 ±0.09 | 0.34 ±0.22 | 0.68 ±0.48 |
| NO (ppm) | 0 | 0.7 ±0.3 | 5.7 ±1.5 | 10.0 ±2.6 |
| $SO_2$ (ppm) | | | | |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | |
| Aliphatic aldehydes (ppm) | | | | |
| Formaldehyde (ppm) | | | | |
| Acrolein (ppm) | | | | |
| Ammonia (ppm) | 1.1 ±3.0 | 1.4 ±1.3 | 0.9 ±0.9 | 0.7 ±0.6 |
| Hydrocarbons (ppm) | 2.6 ±0.6 | 3.8 ±0.9 | 8.7 ±5.2 | 13.4 ±8.3 |
| PAHs Benzo(a)pyrene | | | | |
| Nitropyrene | | | | |

[a] All ± are S.D. unless specified otherwise; data for particles through 30 mo.; data for gases from 35th week through 30 mo.
[b] Mass median diameter.
[c] Geometric standard deviation.
[d] Lovelace multiple jet impactor, mass median aerodynamic diameter.
[e] Impactor/parallel flow diffusion battery, mass median diameter.

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Facility/Sponsor | Inhalation Toxicology Research Institute | | | | | | | |
| Reference | Inhalation Toxicology Research Institute - Annual Report, 1980 | | | | Mauderly et al., 1981[b] | | | |
| Engine type | 1980 GM. 5.7 L | | | | 1980 GM. 5.7 L | | | |
| Operating mode | California 7-mode urban cycle | | | | California 7-mode urban cycle | | | |
| Fuel type | Phillips No. 2 diesel | | | | Phillips No. 2 diesel | | | |
| Fuel sulfur | | | | | | | | |
| Exposure regime | 7 h/d, 5 d/week, 12 weeks | | | | 7 h/d, 5 d/week, 19 weeks | | | |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust | Control | Exhaust | Exhaust | Exhaust |
| Particle conc. ($mg/m^3$) | 0.039 ±0.020 | 0.230 ±0.073 | 1.030 ±0.340 | 4.260 ±1.110 | 0.050 ±0.024 | 0.210 ±0.070 | 1.020 ±0.350 | 4.380 ±1.160 |
| Particle size (μm) MMD[c] (GSD)[d] | | | | | | | | |
| $CO_2$ (%) | | | | 0.2080 ±0.04 | | | | |
| CO (ppm) | 1.1 ±0.6 | 1.5 ±0.6 | 3.7 ±1.1 | 11.5 ±2.6 | | | | |
| $NO_2$ (ppm) | | | | 0.4 ±0.4 | | | | |
| NO (ppm) | | | | 0.80 ±0.25 | | | | |
| $NO_x$ (ppm) | | | | | | | | |
| $SO_2$ (ppm) | | | | | | | | |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | | | | | |
| $O_2$ (%) | | | | | | | | |
| Ozone (ppb) | | | | 14.6 ±3.1 | | | | |
| Aliphatic aldehydes | | | | | | | | |
| Formaldehyde (ppm) | | | | | | | | |
| Acrolein (ppm) | | | | | | | | |
| Ammonia | 2.8 ±0.7 | 3.2 ±0.8 | 2.9 ±0.9 | 2.5 ±0.7 | | | | |
| Hydrocarbons (ppm) | | | | 4.0 ±0.8 | | | | |
| HTHC (ppm) | | | | | | | | |
| PAHs Benzo(a)pyrene | | | | | | | | |
| Nitropyrene | | | | | | | | |

[a] All ± are S.D. unless specified otherwise.
[b] Concentrations of gaseous components reported to be proportional to those in 12-week study
[c] Mass median diameter.
[d] Geometric standard deviation

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| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | |
|---|---|---|---|---|---|
| Facility/Sponsor | Japan Automobile Research Institute Inc. (Health Effects Research Program - HERP) | | | | |
| Reference | HERP, 1988; Ishinishi et al., 1986; Ishinishi et al., 1989 | | | | |
| Engine type | Heavy duty, 11 L, 6-cylinder, direct injection | | | | |
| Operating mode | 1200 rpm, eddy current dynamometer | | | | |
| Fuel type | Nippon Oil Co JIS No. 1 or 2 | | | | |
| Fuel sulfur | 0.41% | | | | |
| Exposure regime | 16 h/d, 6 d/week, 30 mo. | | | | |
| Exposure conditions | Control | Exhaust, filtered | Exhaust | Exhaust, filtered | Exhaust |
| Particle conc. ($mg/m^3$) | 0.004 | 0.005 | 0.39 | 0.019 | 2.99 |
| Particle size ($\mu m$) MMD[b] (GSD)[c] | | | | | 0.31-0.35 (2.58-2.83) |
| $CO_2$ (%) | 0.068 | 0.083 | 0.084 | 0.391 | 0.412 |
| CO (ppm) | 0.06 | 2.54 | 2.50 | 13.00 | 12.90 |
| $NO_2$ (ppm) | 0.024 | 0.42 | 0.44 | 3.96 | 4.95 |
| NO (ppm) | 0.040 | 5.16 | 5.37 | 32.81 | 31.50 |
| $NO_x$ (ppm) | 0.062 | 5.58 | 5.81 | 36.76 | 36.45 |
| $SO_2$ (ppm) | 0.03 | 0.96 | 0.98 | 4.50 | 4.03 |
| $SO_4^{-2}$ ($\mu g/m^3$) | 0.35 | 1.43 | 57.7 | 1.61 | 358 |
| $O_2$ (%) | 20.8 | 20.7 | 20.7 | 20.4 | 20.3 |
| Aliphatic aldehydes | | | | | |
| Formaldehyde (ppm) | 0.003 | 0.04 | 0.04 | 0.24 | 0.20 |
| Acrolein (ppm) | | | | | |
| $NH_4^+$ | | | | | |
| LTHC (ppm) | 3.62 | 4.43 | 4.41 | 7.79 | 7.68 |
| HTHC (ppm) | 2.38 | 3.74 | 4.53 | 12.68 | 13.79 |
| PAHs<br>Benzo(a)pyrene | | | | | |
| Nitropyrene | | | | | |

[a] All ± are S.D unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| Facility/Sponsor | Fraunhofer Institut fur Toxikologie und Aerosolforschung | | | | |
|---|---|---|---|---|---|
| Reference | Heinrich et al. 1982 | | Heinrich et al., 1986, Stober, 1986 | | |
| Engine type | 2.4 L | | 1.6 L | | |
| Operating mode | Constant load of 16kW, 2400 rpm | | FTP (1972) | | |
| Fuel type | European reference fuel | | European reference fuel | | |
| Fuel sulfur | 0.36% | | 0.36% | | |
| Exposure regime | 7-8 h/d, 5 d/week, 104 weeks | | 19 h/d, 6 d/week, 120-140 weeks | | |
| Exposure conditions | Exhaust | Exhaust, filtered | Control | Exhaust | Exhaust, filtered |
| Particle conc. ($mg/m^3$) | 3.9 ±0.5 | | | 4.24 ±1 42 | |
| Particle size (µm) MMD[b] | 0.1 | | | 0.35 ±0.10 | |
| $CO_2$ (%) | 0.54 ±0.15 | 0.52 ±0.13 | 0.10 ±0.01 | 0.38 ±0.05 | 0.35 ±0.05 |
| CO (ppm) | 18.5 ±4.9 | 18.0 ±4 4 | 0.16 ±0.27 | 12.5 ±2.18 | 11.1 ±1.92 |
| $NO_2$ (ppm) | 1.2 ±1.7 | 1.0 ±1.5 | - | 1.5 ±0.33 | 1.2 ±0.26 |
| NO (ppm) | 16.5 ±5.8 | 17.2 ±5.9 | - | 10.0 ±2.09 | 8 7 ±1.84 |
| $NO_x$ (ppm) | 18.6 ±5.8 | 19.2 ±6.1 | - | 11.4 ±2.09 | 9 9 ±1.80 |
| $SO_2$ (ppm) | 3.1 ±1.8 | 2.8 ±1 7 | - | 1.12 ±0.89 | 1.02 ±0.62 |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | | |
| $O_2$ (vol%) | 19.5 ±0 6 | 20 0 ±0 7 | | | |
| Aliphatic aldehydes | | | | | |
| Formaldehyde (ppm) | | | | | |
| Acrolein (ppm) | | | | | |
| $NH_4^+$ | | | | | |
| THC (ppm) | 9.3 ±4 6 | 7 9 ±3.3 | 3.5 ±0.29 | 5.5 ±0.69 | 5.2 ±0.65 |
| $CH_4$ (ppm) | 3.0 ±2.2 | 2.6 ±1.8 | 2.3 ±0.17 | 2.6 ±0.19 | 2.4 ±0 20 |
| PAHs (µg/g part.): Benzo(a)pyrene | 7 0 | | | 3 (13 $ng/m^3$) | |
| Benzo(e)pyrene | 14.1 | | | - (21 $ng/m^3$) | |
| Benz(a)anthracene | 9.8 | | | | |
| Fluoranthene | 134.6 | | | | |
| Pyrene | 65.8 | | | | |
| Benzo(a)fluoranthene | 5.4 | | | | |
| Benzo(b)fluoranthene | 5.3 | | | - (51 $ng/m^3$) | |
| Benzo(ghi)perylene | 21 4 | | | | |
| Coronene | 25.7 | | | | |

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Facility/Sponsor | Fraunhofer Institut fur Toxikologie und Aerosolforschung | | | | | | |
| Reference | Heinrich et al., 1979; Meiss et al., 1981 | | | | | | |
| Engine type | 2.4 L | | | | | | |
| Operating mode | Constant load of 16 kW, 2400 rpm | | | | | | |
| Fuel type | European reference fuel | | | | | | |
| Fuel sulfur | 0.36% | | | | | | |
| Exposure regime | 7-8 h/d, 5 d/week, 5 mo. | | | | | | |
| Exposure conditions | Control | Exhaust | Exhaust, filtered | Exhaust | Exhaust, filtered | Exhaust | Exhaust, filtered |
| Particle conc. (mg/m$^3$) | | 4 | | 11 | | 17 | |
| Particle size (µm)[b] | | 0.1 | | 0.1 | | 0.1 | |
| $CO_2$ (%) | 0.1 | 0.5 | 0.5 | 0.9 | 0.95 | 1.4 | 1.6 |
| CO (ppm) | <1 | 11 | 11 | 25 | 27 | 42 | 45 |
| $NO_2$ (ppm) | | 0.6 | 0.5 | 1.5 | 1.3 | 2.6 | 2.[illegible] |
| NO (ppm) | | 25 | 22 | 43 | 43 | 75 | 68 |
| $NO_x$ (ppm) | | 26 | 23 | 45 | 44 | 78 | 71 |
| SO (ppm) | <1 | 3 | 4 | 8 | 8 | 13 | 12 |
| $SO_4^{-2}$ (µg/m$^3$) | | | | | | | |
| $O_2$ (vol%) | | | | | | | |
| Aliphatic aldehydes | | | | | | | |
| Formaldehyde (ppm) | | | | | | | |
| Acrolein (ppm) | | | | | | | |
| $NH_4^+$ | | | | | | | |
| THC (ppm) | 6 | 8 | 8 | 11 | 12 | 13 | 13 |
| $CH_4$ (ppm) | | 5 | 5 | 5 | 5 | 5 | 5 |
| PAHs<br>Benzo(a)pyrene | | | | | | | |
| Nitropyrene | | | | | | | |

[a] Values estimated from graphically depicted data.
[b] Aerodynamic diameter of the modal peak of the particle mass distribution

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| | | | | | |
|---|---|---|---|---|---|
| Facility/Sponsor | Southwest Research Institute | | | | |
| Reference | Kaplan et al., 1983, White et al., 1983 | | | | Kaplan et al., 1982 |
| Engine type | 5.7 L | | | | 5.7 L |
| Operating mode | Steady state, 1347 rpm, equivalent to constant 40 mph | | | | Steady state, 40 mph |
| Fuel type | Emissions 2D | | | | |
| Fuel sulfur | 0.23-0.24% | | | | |
| Exposure regime | 20 h/d, 7 d/week, 65 weeks | | | | 20 h/d, 7 d/week, 12-13 weeks |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust | Exhaust |
| Particle conc. (mg/m$^3$) | 0.01 ±0.009 | 0.242 ±0.049 | 0.735 ±0.084 | 1.500 ±0.136 | 1.500 |
| Particle size (μm) | | 88-93% < 1.0<br>79-85% < 0.5 | 88-94% <1.0<br>76-84% <0.5 | 91-94% <1.0<br>81-85% <0.5 | |
| $CO_2$ (%) | 0.0649 ±0.0020 | 0.0781 ±0.0028 | 0.1026 ±0.0043 | 0.1355 ±0.0062 | |
| CO (ppm) | 5.81 ±0.2 | 6.39 ±0.3 | 7.43 ±0.3 | 9.40 ±0.5 | |
| $NO_2$ (ppm) | | | | | |
| NO (ppm) | 0 | 0.56 | 1.69 | 3.42 | |
| $NO_x$ (ppm) | 0.05 ±0.0 | 0.65 ±0.1 | 1.85 ±0.2 | 3.73 ±0.4 | |
| $SO_2$ (ppm) | | | | | |
| $SO_4^{-2}$ (μg/m$^3$) | | | | | |
| $O_2$ (%) | | | | | |
| Aliphatic aldehydes | | | | | |
| Formaldehyde (ppm) | | | | | |
| Acrolein (ppm) | | | | | |
| $NH_4^+$ | | | | | |
| Hydrocarbons (ppm) | 3.43 ±0.2 | 3.76 ±0.3 | 4.31 ±0.3 | 4.99 ±0.3 | |
| PAHs<br>Benzo[a]pyrene | | | | | |
| Nitropyrene | | | | | |

[a] All ± are S.D. unless specified otherwise.

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| Facility/Sponsor | Battelle-Geneva Research Center | | | | | Japan Anti-Tuberculosis Association | | |
|---|---|---|---|---|---|---|---|---|
| Reference | Brightwell et al., 1986; Bernstein et al., 1984 | | | | | Iwai et al., 1986 | | |
| Engine type | 1.5 L | | | | | 2.37 L | | |
| Operating mode | FTP - 1972 | | | | | Steady state, 1000 rpm | | |
| Fuel type | | | | | | | | |
| Fuel sulfur | | | | | | | | |
| Exposure regime | 16 h/d, 5 d/week, 104 weeks | | | | | 8 h/d, 7 d/week, 96 weeks | | |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust | Exhaust, filtered | Control | Exhaust, filtered | Exhaust |
| Particle conc. ($mg/m^3$) | | 0.7 | 2.2 | 6.6 | | | | 4.9 ±1.6 |
| Particle size ($\mu m$) MMD[b] (GSD)[c] | | | | | | | | |
| $CO_2$ (%) | | | | 0.46 ±0.03[e] | 0.47 ±0.03[e] | | | |
| CO (ppm) | 1 ±3 | | | 32 ±11 | 32 ±11 | | 7.0 ±1.4[d] | 7.0 ±1.4[d] |
| $NO_2$ (ppm) | | | | | | | 1.8 ±1.8[d] | 1.8 ±1.8[d] |
| NO (ppm) | | | | 5.8 ±2.0[e] | 6.0 ±2.0[e] | | | |
| $NO_x$ (ppm) | 0.1 ±0.1 | | | 8 ±1 | 8 ±2 | | 30.9 ±10.9[d] | 30.9 ±10.9[d] |
| $SO_2$ (ppm) | | | | | | | 13.1 ±3.6[d] | 13.1 ±3.6[d] |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | | | | | |
| $O_2$ (%) | | | | | | | | |
| Aliphatic aldehydes | | | | | | | | |
| Formaldehyde (ppm) | | | | | | | | |
| Acrolein (ppm) | | | | | | | | |
| $NH_4^+$ | | | | | | | | |
| Hydrocarbons (ppm) | | | | 18.9 ±4.1[e] | 18.8 ±4.1[e] | | | |
| PAHs<br>Benzo(a)pyrene | | | | | | | | |
| Nitropyrene | | | | | | | | |

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation.
[d] Samples from dilution tunnel, exposure chamber reported to have approximately the same concentrations.
[e] Data from first year of study (Bernstein et al., 1984).

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | |
|---|---|---|---|---|
| Facility/Sponsor | Battelle, Pacific Northwest Laboratory | | | |
| Reference | Karagianes et al., 1981 | | | |
| Engine type | 43 bhp, 3 cylinder | | | |
| Operating mode | Simulated mining cycle | | | |
| Fuel type | Equivalent to VV-F-800 A grade DF-2 | | | |
| Fuel sulfur | - | | | |
| Exposure regime | 6 h/d, 5 d/week, 87 weeks | | | |
| Exposure conditions | Control | Exhaust | Exhaust + coal dust | |
| Particle conc. ($mg/m^3$) | - | 8.3 ±2.0 | 13.5 ±4.0 | |
| Resp particles ($mg/m^3$) | | 95% respirable | | |
| Particle size (μm) MMD[b] (GSD)[c] | | 0.71 (2.3) | | |
| $CO_2$ (%) | | | | |
| CO (ppm) | | 50 ±3 | | |
| $NO_2$ (ppm) | | 4-6 | | |
| NO (ppm) | | | | |
| $NO_x$ (ppm) | | | | |
| $SO_2$ (ppm) | | <1 | | |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | |
| $O_2$ (%) | | | | |
| Aliphatic aldehydes (ppm) | | <1 | | |
| Formaldehyde (ppm) | | | | |
| Acrolein (ppm) | | | | |
| Ammonia (ppm) | | 26-40 | | |
| Hydrocarbons (ppm) | | | | |
| PAHs<br>Benzo(a)pyrene | | | | |
| Nitropyrene | | | | |

[a] All ± are S.D. unless specified otherwise.
[b] Mass median diameter.
[c] Geometric standard deviation

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| Facility/Sponsor | University of Pittsburgh | | | National Board of Occupational Safety and Health - Sweden | Ministry of Supply Chemical Defense Experimental Establishment | | | |
|---|---|---|---|---|---|---|---|---|
| Reference | Battigelli, 1965 | | | Ulfvarson et al., 1987 | Pattle et al., 1957 | | | |
| Engine type | 7 hp, four cycle, single cylinder | | | 1980 Volvo, 6 cylinder | 0.568 L, single cylinder | | | |
| Operating mode | | | | 2,500 rpm | 1,600 rpm; A - no load; B - load; C - load plus worn injector; D - no load, high fuel-air ratio. | | | |
| Fuel type | | | | | 47 cetane | | | |
| Fuel sulfur | | | | | 0.51% | | | |
| Exposure regime | 15-60 min | | | 3 h, 40 min | 5 h | | | |
| Exposure conditions | Dilution A | Dilution B | Dilution C | Exhaust | A | B | C | D |
| Particle conc. ($mg/m^3$) | | | | 0.6 | 74 | 122 | 53 | 1,070 |
| Particle size ($\mu m$) | | | | | | | | |
| $CO_2$ (%) | 0.1 | 0.9 | 1.1 | | | | | |
| CO (ppm) | <20 | 30 | 55 | 4.63 | 560 | 410 | 380 | 1,700 |
| $NO_2$ (ppm) | 1.3 | 2.8 | 4.2 | 2.07 | 23 | 51 | 43 | 12 |
| NO (ppm) | | | | 4.56 | | | | |
| $NO_x$ (ppm) | | | | | <6 | 209 | 174 | 44 |
| $SO_2$ (ppm) | 0.2 | 0.5 | 1 | | | | | |
| $SO_4^{-2}$ ($\mu g/m^3$) | | | | | | | | |
| $O_2$ (%) | 20.5 | 20.0 | 19.5 | | | | | |
| Aliphatic aldehydes | <1.0 | <1-2 | 1-2 | | 16[b] | 6.0[b] | 6.4[b] | 154[b] |
| Formaldehyde (ppm) | <0.1 | <0.1 | <0.1 | 0.04 | | | | |
| Acetaldehyde | | | | 0.17 | | | | |
| Acrolein (ppm) | <0.05 | <0.05 | <0.05 | | | | | |
| $NH_4^+$ | | | | | | | | |
| Hydrocarbons (ppm) | <2.0 | 2.5 | 3.2 | | | | | |
| Benzene (ppm) | | | | 0.06 | | | | |
| Toluene (ppm) | | | | 0.35 | | | | |
| PAHs ($\mu g/m^3$): Benzo(a)pyrene | | | | 640 | | | | |
| Nitropyrene | | | | | | | | |

[a] All ± are S.D. unless specified otherwise
[b] As formaldehyde

## APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a]

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Facility/Sponsor | U.S. Environmental Protection Agency | | | | | | | |
| Reference | Gillespie, 1980; Hyde et al., 1980; Malanchuk, 1980; Orthoefer, 1980; Stara et al., 1980 | | | | | | | |
| Engine type | Automobile gasoline engine | | | | | | | |
| Operating mode | Urban cycle | | | | | | | |
| Fuel type | | | | | | | | |
| Fuel sulfur | | | | | | | | |
| Exposure regime | 16 h/d, 7 d/week, 68 mo. | | | | | | | |
| Exposure conditions | Control | Non-irradiated gasoline exhaust (R) | Irradiated gasoline exhaust (I) | $SO_2$ + $H_2SO_4$ | R + $SO_2$ + $H_2SO_4$ | I + $SO_2$ + $H_2SO_4$ | Nitrogen oxides | Nitrogen oxides |
| Particle conc. ($mg/m^3$) | | | | | | | | |
| Particle size (μm) | | | | | | | | |
| $CO_2$ (%) | | | | | | | | |
| CO (ppm) | 4.9 | 97.5 ±10.0 | 94.5 ±19.6 | - | 98.4 ±13.8 | - | - | - |
| $NO_2$ (ppm) | 0.04 | 0.05 ±0.02 | 0.94 ±0.36 | - | 0.05 ±0.03 | 0.89 ±0.36 | 0.64 ±0.12 | 0.15 ±0.33 |
| NO (ppm) | 0.04 | 1.45 ±0.42 | 0.19 ±0.29 | - | 1.51 ±0.44 | 0.19 ±0.29 | 0.25 ±0.06 | 1.67 ±0.21 |
| $NO_x$ (ppm) | | | | | | | | |
| $SO_2$ (ppm) | 0.03 | - | - | 0.42 ±0.22 | 0.48 ±0.23 | 0.42 ±0.21 | - | - |
| $H_2SO_4$ (ppm) | - | - | - | 0.02 ±0.01 | 0.02 ±0.01 | 0.03 ±0.01 | - | - |
| Oxidants (ppm as $O_3$) | 0.02 | - | 0.20 ±0.09 | - | - | 0.20 ±0.08 | - | - |
| Aliphatic aldehydes | | | | | | | | |
| Formaldehyde (ppm) | | | | | | | | |
| Acrolein (ppm) | | | | | | | | |
| $NH_4^+$ | | | | | | | | |
| Hydrocarbons (ppm as $CH_4$) | 2.7 | 27.5 ±4.4 | 23.9 ±6.1 | - | 27.4 ±4.3 | 23.9 ±6.0 | - | - |
| PAHs | | | | | | | | |
| Benzo(a)pyrene | | | | | | | | |
| Nitropyrene | | | | | | | | |

[a] All ± are S.D. unless specified otherwise.

| APPENDIX A. EXPERIMENTAL PROTOCOL AND COMPOSITION OF EXPOSURE ATMOSPHERES[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Facility/Sponsor | Japan Automobile Research Institute Inc. (Health Effects Research Program - HERP) | | | | | | | | | |
| Reference | HERP 1988; Ishinishi et al., 1986; Ishinishi et al., 1989 | | | | | | | | | |
| Engine type | Light duty, 1.8 L, 4-cylinder, swirl chamber | | | | | Heavy duty, 11 L, 6-cylinder, direct injection | | | | |
| Operating mode | 1700 rpm, eddy current dynamometer | | | | | 1200 rpm, eddy current dynamometer | | | | |
| Fuel type | Nippon Oil Co JIS No 1 or 2 diesel | | | | | Nippon Oil Co JIS No. 1 or 2 diesel | | | | |
| Fuel sulfur | 0.41% | | | | | 0.41% | | | | |
| Exposure regime | 16 h/d, 6 d/week, 30 mo. | | | | | 16 h/d, 6 d/week, 30 mo. | | | | |
| Exposure conditions | Control | Exhaust | Exhaust | Exhaust | Exhaust | Control | Exhaust | Exhaust | Exhaust | Exhaust |
| Particle conc. ($mg/m^3$) | 0.003 | 0.11 | 0.41 | 1.08 | 2.32 | 0.002 | 0.46 | 0.96 | 1.84 | 3.72 |
| Particle size (μm) MMD[b] (GSD)[c] | | | | 0.19 (2.37-2.71) | 0.21-0.22 (2.23-2.93) | | | | 0.20-0.23 (2.73-3.07) | 0.25-0.28 (2.75-3.18) |
| $CO_2$ (%) | 0.026 | 0.050 | 0.105 | 0.219 | 0.418 | 0.035 | 0.084 | 0.140 | 0.215 | 0.360 |
| CO (ppm) | 0.80 | 1.23 | 2.12 | 3.96 | 7.10 | 0.63 | 2.65 | 4.85 | 7.75 | 12.91 |
| $NO_2$ (ppm) | 0.011 | 0.08 | 0.26 | 0.70 | 1.41 | 0.021 | 0.46 | 1.02 | 1.68 | 3.00 |
| NO (ppm) | 0.033 | 1.16 | 3.81 | 9.44 | 18.93 | 0.042 | 5.71 | 12.11 | 19.99 | 34.45 |
| $NO_x$ (ppm) | 0.044 | 1.24 | 4.06 | 10.14 | 20.34 | 0.061 | 6.17 | 13.13 | 21.67 | 37.45 |
| $SO_2$ (ppm) | 0.06 | 0.38 | 1.06 | 2.42 | 4.70 | 0.06 | 0.98 | 1.79 | 2.82 | 4.57 |
| $SO_4^{-2}$ ($\mu g/m^3$) | 0.41 | 18.8 | 62.4 | 151 | 315 | 0.49 | 62.9 | 111 | 198 | 361 |
| $O_2$ (%) | 20.8 | 20.8 | 20.7 | 20.5 | 20.3 | 20.8 | 20.8 | 20.7 | 20.6 | 20.4 |
| Aliphatic aldehydes | | | | | | | | | | |
| Formaldehyde (ppm) | 0.002 | 0.01 | 0.03 | 0.07 | 0.13 | 0.003 | 0.05 | 0.11 | 0.18 | 0.29 |
| Acrolein (ppm) | | | | | | | | | | |
| $NH_4^+$ | | | | | | | | | | |
| Hydrocarbons (ppm) | 2.17 | 2.27 | 2.51 | 2.87 | 3.57 | 3.50 | 4.27 | 5.16 | 5.90 | 7.62 |
| HTHC (ppm) | 2.20 | 2.44 | 2.93 | 3.82 | 5.49 | 2.43 | 4.63 | 7.15 | 9.94 | 15.65 |
| PAHs ($ng/m^3$) Benzo(a)pyrene | | | | | 5.3 ±10.6 | | | | | 7.5 ±3.2 |
| Benzo(k)fluoranthene | | | | | 5.4 ±7.7 | | | | | 6.0 ±3.0 |
| Benzo(ghi)perylene | | | | | 2.7 ±3.9 | | | | | 8.9 ±2.5 |
| 1-Nitropyrene | | | | | 46.6 ±44.0 | | | | | 43.4 ±9.8 |

[a] All ± are S.D., unless specified otherwise
[b] Mass median diameter.
[c] Geometric standard deviation

# APPENDIX B

# CONTRACTOR REPORT: ASSESSMENT OF RISK FROM EXPOSURE TO DIESEL ENGINE EMISSIONS

## SUMMARY

Significant numbers of diesel locomotives began to appear on U.S. railroads in the 1940s, and by 1959, virtually all U.S. locomotives were powered by diesel engines. This report describes an effort to conduct a quantitative assessment of the risk of lung cancer from exposure to diesel exhaust (DE) based upon data from a retrospective cohort study of lung cancer mortality among U.S. railroad workers (Garshick *et al.*, 1988) and an industrial hygiene study of exposures of U.S. railroad workers to DE (Woskie *et al.*, 1988a,b; Hammond *et al.*, 1988).

Garshick *et al.* (1988) studied information obtained from the Railroad Retirement Board (RRB) on 55,407 white males who began railroad employment between 1939 and 1949, who were between the ages of 40 and 64 in 1959, and who in 1959 worked at one of 39 jobs selected to represent a range of potential exposures to DE. Garshick *et al.* (1988) reported two analyses that indicated an effect of exposure to DE upon lung cancer risk in this cohort:

1) A relative risk for lung cancer of 1.45 (95% CI = 1.11, 1.89) was observed for DE-exposed workers who were 40-44 years of age in 1945 and who consequently had the longest potential exposure to DE; relative risks were progressively lower among DE-exposed workers who were older in 1959 and who thus had potentially shorter exposures to DE.

2) The relative risk of lung cancer increased monotonically with increasing duration of work in 1959 or later in a job involving diesel exposure (disregarding exposures in the current year and in the four most recent years); this relative risk was 1.72 (95% CI = 1.29, 2.33) in the group with the longest exposure (15-17 years).

Concentrations of respirable particulate matter (RSP) were collected in four small, northern U.S. railroads during 1981-1983 (Woskie *et al.*, 1988a; Hammond *et al.*, 1988).

This information was collected for 13 jobs categories, which were obtained by grouping the 39 jobs used to define the Garshick *et al.* (1988) cohort. In addition to RSP, two additional markers of DE were developed: adjusted respirable particulate (ARP), which is determined by subtracting from the concentration of RSP an estimate of the concentration of environmental tobacco smoke (ETS), and adjusted extractable matter (AEM), which is the concentration of RSP that is extractable by dichloromethane minus an estimate of the concentration of the extractable fraction of ETS present. Significant differences were found between concentrations of ARP in samples collected on warm days (>10°C) and cold days (<10°C). Because of this temperature effect, the concentration of ARP was estimated separately for warm and cold days for each job group.

In the present study, data from Woskie *et al.* (1988a) and Hammond *et al.* (1988) were used to assign average exposures to each cohort member by year, beginning in 1959 based on yearly job codes. In addition to RSP, ARP and AEM, exposure to total extractable material (TEX) was also studied. TEX is the concentration of extractable RSP, not adjusted to remove the contribution of ETS. Since ETS is also suspected of being a risk factor for lung cancer, it is plausible that TEX might be more closely correlated with lung cancer than measures that omit ETS.

Temperature-specific estimates were developed for each of these four markers, and a temperature-weighted estimate of each worker's yearly exposure to each marker was obtained, based on the location of the last railroad where he was employed and temperature data for that location. As a hedge against the possibility that the temperature-specific estimates were biased for some reason, ARP, unadjusted for temperature, was also studied.

Job codes were not available for years prior to 1959, so it was assumed that during those years of employment a person worked in the same job as in 1959. Exposures in each job category were assumed to begin after 1945 and to increase linearly by year through 1959, when dieselization of U.S. railroads was virtually complete.

Poisson regression using internal controls was the principal statistical method used to study the relationship between measures of diesel exposure and lung cancer mortality. Measures of exposure considered were: cumulative exposure omitting the most recent

three years; cumulative exposure 4-8 years in the past; cumulative exposure 9-18 years in the past; cumulative exposure more than 18 years in the past, and maximum exposure in any year prior to the three most recent years. Diesel exposure was also dichotomized as yes/no, with clerks and signalmen considered to be unexposed.

Measures of cumulative exposure during different time intervals in the past were studied in order to investigate different potential latency periods for lung cancer induced by DE, i.e., to determine whether exposures during a particular period in the past had a greater influence on lung cancer mortality than exposures during different periods. If this was the case, then including exposures during other time periods could mask an effect of diesel exposure. Measures based on maximum exposure were considered to explore an hypothesis suggested by animal data that clearance of respirable particles from the lung is capacity limited and, as a result, more intense exposures may be more dangerous per amount inhaled than less intense exposures.

Other explanatory variables considered in the analyses were age, calendar year and job category (clerk; signalman; engineer and firer; brakeman, conductor and hostler; shop worker). Both relative risk models and absolute risk models were applied to the data.

More than 50 analyses of the relationship between exposure to DE and lung cancer mortality were conducted. These involved five markers of exposure to DE, five ways of accumulating previous exposures, several subgroups of the cohort based on job in 1959, and both relative risk and absolute risk models. None of these analyses demonstrated a pattern that was consistent with an adverse effect of diesel upon lung cancer; in fact, many of them showed a statistically significant negative association. Consequently, we were not able to project a dose response for lung cancer induced by DE or estimate the carcinogenic potency of DE from these data.

The data available in Woskie *et al.* (1988a) and Hammond *et al.* (1988) to estimate exposures to DE in the cohort studied by Garshick *et al.* (1988), and the methods applied to these data, have a number of limitations, including:

- Use of markers of exposure that are not specific for DE;
- Lack of personal sampling data for members of the cohort;

- Extrapolating exposures measured in four small railroads in a limited geographical region to the entire U.S.;

- Assuming exposures measured during 1981-1983 were representative of earlier exposures;

- Lack of knowledge of the extent to which individual workers worked with diesel equipment during the period of changeover from steam to diesel;

- Adjusting exposures for temperature differences based on limited information on the area served by the last railroad at which a worker was employed.

Because of these limitations, there is considerable uncertainty regarding the estimates of exposure of the cohort to DE. These uncertainties are potentially of sufficient magnitude to obscure any relationship between exposure to DE and lung cancer that may exist in the cohort. Consequently, these negative findings may be due to weaknesses in the exposure data rather than to an absence of diesel effect, *per se*.

Analyses in which diesel exposure was considered as a yes/no variable confirmed the first finding of Garshick *et al.* (1988) Further analyses in which workers in specific diesel-exposed jobs were compared to unexposed workers revealed that:

- The risk of lung cancer among engineers and firers was significantly elevated relative to that among unexposed railroad workers, and the variation in relative risk with age in 1959 (increasing risk with decreasing age) is consistent with DE being responsible for the observed excess.

- Although the risk of lung cancer among conductors, brakemen and hostlers was significantly elevated relative to that among unexposed railroad workers, the variation in risk with age in 1959 (higher at older and younger ages than at intermediate ages) is not consistent with an effect of DE.

- The risk of lung cancer among shop workers was not significantly elevated relative to that of unexposed railroad workers. This is not

consistent with an effect of DE upon lung cancer because it seems likely that shop workers had significantly higher exposures to DE than either of the other two groups of exposed workers.

An age-specific plot of percentage of deaths in this cohort by calendar year reveals that age-specific death rates for all causes remain fairly constant at values roughly comparable to corresponding rates in U.S. white males until about 1977. Beginning in about that year, death rates begin to decrease year-by-year, and in 1980 they are from two-fold to six-fold smaller than corresponding age-specific rates in U.S. white males. The only plausible explanation for this that we can envision is that a significant number of unrecorded deaths must have occurred in the cohort after 1977. A new tape has recently been provided by the RRB that includes follow-up of the cohort for additional years, as well as an update of the follow-up through 1980 (Garshick, 1991). It is reported that for 1980, about 25% of the deaths on the updated tape were not on the earlier tape. Some additional deaths in 1979 are also on the new tape, but the percentage is much smaller. However, our work suggests that the lack of follow-up may have been more severe than this.

A Poisson regression analysis of cumulative years of work in 1959 or later in a job involving diesel exposure (disregarding exposures in the current year and four most recent years) found an inverse relationship between cumulative years of exposure and relative risk of lung cancer. The relative risk of lung cancer for 1-4 cumulative years of exposure was 1.38 (95% CI = 1.17, 1.62), for 5-9 years of cumulative exposure was 1.28 (95% CI = 1.10, 1.49), for 10-14 years of cumulative exposure was 1.10 (95% CI = 0.92, 1.3), and for 15-17 years of exposure was 1.06 (95% CI = 0.75, 1.50). Although the reason for the differences in these results and those of Garshick *et al.* (1988) has not been completely determined, it appears to be related to the fact that the three variables used in the analysis -- calendar year, age, and cumulative years of exposure -- are all correlated, which makes the analyses highly sensitive to the specific method used for controlling for age and calendar year. Whereas Garshick *et al.* (1988) modelled age as a continuous variable and used calendar year to define the risk sets in a Cox regression

analysis, we controlled for calendar year using an indicator variable for each year and controlled for age using an indicator variable for each five-year age interval.

Follow-up of railroad workers' mortality in this study was through 1980, which is only 22 years since the dieselization of U.S. railroads was essentially complete. Since the time from first exposure until evidence of an increased risk of environmentally induced lung cancer is often on the order of 20 years, the full impact of any effect of DE upon lung cancer in this cohort may not be captured by the current study. Considering this, and also considering the fact that follow-up may have been incomplete in the latter years of the study, it would be worthwhile to conduct a new study of this cohort to take advantage of the several additional years of follow-up now available. If such a study is conducted, it is recommended that vital status be verified independently of RRB records.

## INTRODUCTION

Diesel engines emit a much larger quantity of respirable particles than gasoline engines of comparable size (McClellan, 1987). Associated with these particles are carcinogenic and mutagenic compounds, which suggests that diesel exhaust (DE) may be carcinogenic when inhaled. A considerable amount of research, including epidemiological studies and *in vitro* and whole animal bioassays, have been conducted to evaluate the carcinogenic potential of DE.

Extracts from DE have been shown to be active (e.g., mutagenic) in a number of short-term *in vitro* assays and carcinogenic in SENCAR mouse skin tumor assays (Lewtas and Williams, 1986). Exposure of rats to DE at high levels for a prolonged period has resulted in excess lung tumors (Mauderly et al., 1986).

Howe *et al.* (1983) reported an elevated risk of lung cancer among railroad pensioners who were formerly exposed to DE and coal dust. The study did not contain information on duration or levels of exposure to DE.

Garshick *et al.* (1987) conducted a case control study of lung cancer deaths among U.S. railroad workers. A significantly increased relative odds (odds ratio = 1.41) was observed among men who were 64 years old or younger at the time of death, and who had worked for 20 years or longer in a job involving diesel exposure. No effects of DE

were seen in workers 65 or older; however, it was reported that many of these workers retired shortly after the transition to diesel locomotives was complete.

Garshick *et al.* (1988) reported on a retrospective cohort study of 55,407 U.S. railroad workers who began railroad work in 1950 or earlier and were followed from 1959 through 1980. The findings of this study are summarized in the next section. As part of this study, an industrial hygiene study was conducted between 1981 and 1983 of exposure of U.S. railroad workers to DE (Woskie *et al.*, 1988a,b; Hammond *et al.*, 1988).

A number of other epidemiological studies of diesel-exposed populations have not found an increase in cancer (Kaplan, 1995; Waxweiler *et al.*, 1973; Waller, 1980; Hall and Wynder, 1984; Wong *et al.*, 1985).

The present study describes an effort to make quantitative estimates of the cancer risk posed by exposure to DE using epidemiological data from the Garshick *et al.* (1988) retrospective cohort study of U.S. railroad workers and information on the exposures of these workers to DE from Woskie *et al.* (1988a,b) and Hammond *et al.* (1988).

## DESCRIPTION OF EPIDEMIOLOGICAL DATA

### Review of Study of U.S. Railroad Workers

Using Interstate Commerce Commission (ICC) job codes Garshick *et al.* (1988) identified 39 job groups for study. Job groups selected involved large groups of workers, with and without regular exposure to DE. Major categories of jobs assumed to involve substantial exposure to DE were shop worker, engineer, firer, brakeman, conductor, and hostler. Clerical workers (clerks, station agents, dispatchers, etc.) and signalmen were considered to be unexposed.

The RRB provided data on workers in these job groups based on job codes for 1959. For their analysis, Garshick *et al.* (1988) selected a cohort consisting of 55,407 white males who began their employment between 1939 and 1949 and were between the ages of 40 and 64 in 1959.

The follow-up period for the study was from 1959 through 1980. Workers not reported by the RRB to have died by December 31, 1980, were considered to be alive. Cause-specific death certificates were obtained for 88.3% of the deaths; remaining deaths were assumed to be due to unknown causes.

Partial likelihood methods (Cox, 1972), using calendar year to define risk sets, were used to compare the lung cancer mortality in diesel-exposed and non-diesel-exposed workers. Age was controlled for by estimating category variables for five categories of age in 1959. Also calculated was the relative risk of lung cancer by categories of years of exposure to DE, compared to non-exposure, using age as a continuous variable.

The age-specific relative risk of lung cancer in exposed workers versus unexposed workers was 1.45 (95% CI = 1.11-1.89) for those aged 40-45 and decreasing in an almost monotonic fashion to a relative risk of 0.99 among workers aged 60-64 in 1959. Similar analyses of relative risks among workers potentially occupationally exposed to asbestos versus workers not so exposed did not show a relationship with potential asbestos exposure. The results pertaining to diesel exposure were reproduced by independently calculating directly standardized relative risks for lung cancer among diesel workers versus unexposed workers for each five-year age group in 1959.

Analyses of relative risk versus years of exposure did not find a consistent exposure-response relationship. However, if exposure in the year of death and the four most current years were omitted, the group with $\geq$15 years of exposure had a relative risk of 1.72 (95% CI = 1.29, 2.33). Smaller but statistically significant relative risks were obtained for 1-4, 5-9, and 10-14 years of cumulative exposure. These relative risks increased monotonically with increasing years of exposure.

The higher relative risks among persons who were younger in 1959 are consistent with an effect of DE upon lung cancer because the younger groups would have had a longer time remaining to work and be exposed to DE. Similarly, higher relative risks among persons with longer exposures to diesel are consistent with an effect of DE upon lung cancer.

**Epidemiologic Data Available for This Assessment**

Data on U.S. railroad workers used in the Garshick *et al.* (1988) study were provided to us on tape by Dr. Garshick and Dr. Speizer. Table 1 contains a catalog of the data received. ICC_1 through ICC_22 are the yearly job codes (ICC, 1951) provided to the RRB. A list of the sampled job codes for 1959 used to define the cohort and the number of workers in each category are shown in the top portion of Table 2. Some

workers were assigned job codes in years subsequent to 1959 that were not among those sampled. These unsampled job codes appear in the bottom half of Table 2.

There were 19,384 deaths in the cohort from all causes. Deaths were considered due to lung cancer if the death certificate listed ICC code 162.0 or 162.1 as either the primary or secondary cause of death. There were a total of 1,640 deaths from lung cancer as determined by the primary cause of death and 54 (3%) as determined by the secondary cause of death, for a total of 1,964 deaths from lung cancer.

## DESCRIPTION OF EXPOSURE DATA

Data used to estimate exposure to DE in this cohort come from two published papers concerning measurements of markers for diesel exposure collected from four small railroads in the northern U.S. between 1981 and 1983 (Woskie *et al.*, 1988a; Hammond *et al.*, 1988). Measuring exposure to DE is complicated by the fact that diesel particulate is a complex mixture containing respirable carbonaceous particles upon which many organic chemicals may be adsorbed. Because of the complex chemical nature of diesel particulate, it is very difficult to distinguish diesel particulate from respirable particulate matter from other sources.

To deal with this problem, Woskie *et al.* (1988a) and Hammond *et al.* (1988) considered several markers for diesel particulate in addition to the concentration of total respirable particulate. Woskie *et al.* proposed adjusted respirable particulate (ARP) concentration as a marker for diesel exposure. ARP is estimated by subtracting from the concentration of respirable particulate matter (RSP) an estimate of the concentration of environmental tobacco smoke (ETS). In addition, Hammond *et al.* proposed the concentration of adjusted extractable matter (AEM) as a marker for DE. AEM is defined as the concentration of RSP that is extractable by dichloromethane minus an estimate of the extractable fraction of ETS. Railroad workers can be exposed to inorganic respirable particles from a variety of sources, including welding material, fibrous material and aerosolized sand (Hammond *et al.*, 1988). Use of AEM as a marker for diesel particulate can help eliminate interference from respirable inorganic particulate matter.

The 39 job codes sampled by Garshick *et al.* (1988) were combined into 13 homogeneous job groups and over 550 samples of RSP were collected in these 13 job groups. The job groups were selected so that exposures within a group represented essentially the same type of work and proximity to running locomotives. Each sample was collected over a single work shift. Personal breathing zone samples were collected from all job categories except clerk and engineer. Area samples were collected for these latter jobs, as workers in these jobs were considered to remain essentially stationary. Each sample was categorized according to outdoor temperature as warm (mean outdoor temperature > 10°C) or cold (mean temperature < 10°C).

Table 1
Contents of Data Tape on Mortality of U.S. Railroad Workers Used in Analysis*

| Variable Name | Description |
|---|---|
| IDENT | Unique identification number |
| AGE59 | Age in 1959, 40-64 |
| ASB59 | Potential for asbestos exposure based on job code in 1959, defined as HI or NO |
| BANO | Last railroad employer |
| COD1 | Underlying cause of death from the death certificate based on ICDA-8 |
| COD2 | "Secondary" cause of death, coded only if cancer or chronic lung disease was noted on the death certificate and it was not the underlying cause of death |
| DEATHL12 | 1=lung cancer death, COD1 or COD2, 0=death not due to lung cancer |
| FIRST_ES | Estimated year first worked for the railroad, based on total service months and year last worked for the railroad |
| FIRWRK | Year first worked for the railroad, provided by the Railroad Retirement Board, but exists only for 1947 or greater |
| ICC_1 to ICC_22 | Yearly 3 digit job codes, 1959-1980 |
| EXPOS_1 to EXPOS_22 | Yearly job category, 1959-1980 |
| MODC | Month of death |
| MON_1 to MON_22 | Months of railroad service by year, 1959-1980 |
| MSURV59 | Survival in months from January 1959 until death or end of the follow-up period |
| RACE | All white males; RACE=1 |
| RET | Retirement year (year last worked for the railroad) |
| SRVC | Months of total railroad service |
| SSN | Social security number (dead cases only) |
| YODH | Year of death |
| YOBH | Year of birth |

*Provided by Dr. Garshick.

Table 2
Job Codes for Cohort
Garshick et al. (1988) Exposure Group

| Job Category | ICC Codes | Number of Men |
|---|---|---|
| **A. SAMPLED JOB CODES IN 1959 (Used to define cohort)** | | |
| Clerk | 007,012,075,076,078-083 | 10,475 |
| Signalman | 044-049 | 3,548 |
| Passenger Engineer/Fireman | 121,125 | 881 |
| Yard Engineer/Fireman | 124,128 | 4,446 |
| Freight Engineer/Fireman | 122,123,126,127 | 5,676 |
| Passenger Brakeman/Conductor | 111,112,116 | 1,148 |
| Freight Brakeman | 117,118 | 6,134 |
| Freight Conductor | 113,114 | 1,089 |
| Yard Brakeman/Conductor | 119,120 | 9,126 |
| Hostler | 108-110 | 780 |
| Machinist | 061 | 6,635 |
| Electrician | 058 | 4,288 |
| Supervisor (Shop) | 050 | 1,169 |
| **B. UNSAMPLED JOB CODES** | | |
| Clerk | 001-006,008-011,013-016,019-022,026,077, 084-087,099,104-106,129,301-311,317-318 | |
| Signalman | 017-018,024,028,035-043,073,074,088-094, 098,102-103,312-316,319-325 | |
| Shop Workers | 052-055,062-066,068,070-071 | |
| Brakeman | 107 | |
| Other Train Riders | 095-097,100-101,115 (for 115, EXPOS assigned as 6) | |
| Carman | 023,025,027,029-034,051,056, 057,059,060, 067,069,072,390-395,200,202-204,211 | |
| Unknown Job | 000 | |
| No Job/After Retirement | no ICC Code present in array | |

A cyclone was used to insure collection of respirable particles only. The amount of respirable particulate on each filter was determined as the difference in the filter weight prior to and subsequent to collection of the sample.

After determining the amount of RSP from each filter, the filters from three of the railroads were composited by job group and railroad and extracted with dichloromethane. One-half of the extracted material was analyzed by gas chromatograph to determine the amount of nicotine present. The concentration of ETS in each pooled sample was estimated by comparing the amount of nicotine in each extract to the amount of nicotine in experimental samples of RSP known to be composed solely of ETS.

Information on ETS was not available for one of the four railroads or for some of the job categories in the remaining three railroads. The average estimated concentration within a job group was used to estimate the average concentration for the missing data in that group. The concentration of ARP was estimated for each job group across all railroads by subtracting the applicable average fraction that was due to ETS from each railroad's average RSP concentration. A weighted average concentration for each job category was calculated using the number of samples from each railroad as the weights.

The half of extracted material not used for nicotine determination was combined across the three railroads by job category and weighed to determine the amount of extractable material present. The concentration of AEM was then calculated for each job category as the concentration of RSP times the fraction extractable minus the estimated concentration of ETS times the fraction of ETS extractable.

Table VII of Woskie *et al.* (1988a) contains average concentrations of AEM for each of 13 job groups, categorized by "Cold" and "Warm". For some jobs there are significant differences between concentrations for "Cold" and "Warm" days. Exposures in the indoor jobs of clerks and shop workers are about twice as high on cold days as on warm days, which is consistent with improved ventilation on warm days. On the other hand, other jobs involve higher exposures on warm days.

**Development of Measures of Diesel Exposure for U.S. Railroad Workers**

**Development of Temperature-Specific Estimates of Exposure.** The differences in average concentrations of AEM on cold and warm days obtained by Woskie *et al.*, suggest that estimates of individual exposures could be improved by taking temperature

differences into account, based upon the geographical area in which a cohort member worked. The first step in accomplishing this was to develop temperature-specific estimates of exposure for exposure measures other than ARP. Table II of Hammond *et al.* contains estimates of average concentrations of RSP and AEM obtained from three railroads. These estimates were made temperature-specific by multiplying a concentration of RSP or AEM from Hammond *et al.*'s Table II by the ratio of the temperature-specific concentration of ARP from Woskie *et al.*'s Table VII to the corresponding (temperature independent) estimate for ARP from Hammond *et al.*'s Table II.

In addition, a fourth marker of exposure was developed called total extractable material (TEX). Temperature-specific estimates of TEX were developed by multiplying the percent extractable mass from Hammond *et al.*'s Table III by the corresponding RSP concentration in Hammond *et al.*'s Table II and then making the estimates temperature-specific as described in the previous paragraph. RSP contains material other than diesel particulate that may be carcinogenic to the lung (e.g., tobacco smoke), so it is of interest to determine the extent to which TEX is a predictor of lung cancer incidence.

The resulting temperature- and job-specific exposure estimates are tabulated in Table 3. Estimating the temperature-specific values for the other markers from temperature-specific values for ARP is a relatively crude approach, because temperature gradients in exposures may be different for different exposure markers. However, this approach provides a reasonable approximation and, unfortunately, temperature-specific data are not available for the other exposure markers of interest.

In order to use the temperature-specific estimates of exposure, temperature variables were developed for each member of the cohort to reflect the prevailing temperature patterns in the area in which an individual worked. The only railroad-specific information available for members of the cohort was a code indicating the name of the last railroad for which the person had worked. It was assumed that the temperature patterns in the location served by this railroad would be representative of his entire work history.

Each railroad has designated one or more individuals as contact officials who interacted with the RRB on various matters. Upon request, the RRB furnished the

names and address of the contact person for each railroad. These addresses were used to assign a geographical area (state) to each railroad. In some cases the area of operation was ambiguous and "The Official Guide of the Railways, May, 1970" was used to designate a state of operation of a railroad. According to information obtained from the RRB, there are a limited number of railroads that operate in multiple geographic regions (Class I railroads) and the operation of most other railroads is confined to the immediate area surrounding their headquarters (Ferguson, 1990).

A publication of the National Oceanic and Atmospheric Administration (NOAA, 1978) contains climatological information for various U.S. cities by month, including the average temperature, average maximum daily temperature and average percent of days that were clear, cloudy or partly cloudy, based on data from 1941 through 1970. Data from a centrally located city in each state were selected as representative of climatological data for that state.

Table 3
Climate Adjusted Exposures ($\mu g/m^3$)

| | | ARP | | AEM | | RSP | | TEX | |
|---|---|---|---|---|---|---|---|---|---|
| Job Description | ARP | Cold | Warm | Cold | Warm | Cold | Warm | Cold | Warm |
| Clerk | 42 | 54 | 25 | 9.3 | 4.3 | 167.1 | 77.4 | 81.9 | 37.9 |
| Signalman | 58 | 33 | 74 | 13.1 | 29.3 | 40.4 | 90.6 | 16.6 | 37.1 |
| Passenger Engineer/ Fireman | 51 | 18 | 68 | 8.5 | 32.0 | 43.1 | 162.7 | 17.7 | 66.7 |
| Yard Engineer/Fireman | 69 | 65 | 72 | 15.1 | 16.7 | 69.7 | 77.2 | 28.6 | 31.7 |
| Freight Engineer/ Fireman | 94 | 108 | 67 | 34.5 | 21.4 | 114.9 | 71.3 | 47.1 | 29.2 |
| Passenger Brakeman/ Conductor | 104 | 79 | 123 | 20.5 | 31.9 | 85.1 | 132.5 | 23.8 | 37.1 |
| Freight Brakeman | 102 | 110 | 94 | 8.5 | 7.3 | 138.0 | 118.0 | 31.7 | 27.1 |
| Freight Conductor | 69 | 80 | 52 | 34.8 | 22.6 | 148.4 | 96.5 | 69.8 | 45.3 |
| Yard Brakeman/Conductor | 114 | 150 | 56 | 22.4 | 8.4 | 252.6 | 94.3 | 78.3 | 29.2 |
| Hostler | 224 | 276 | 172 | 40.7 | 25.3 | 287.1 | 178.9 | 45.9 | 28.6 |
| Machinist | 147 | 181 | 95 | 44.0 | 30.0 | 231.0 | 127.0 | 73.9 | 53.3 |
| Electrician | 194 | 257 | 113 | 79.0 | 32.0 | 254.0 | 118.0 | 109.2 | 50.7 |
| Supervisor (Shop) | 155 | 160 | 40 | 44.4 | 11.1 | 250.8 | 62.7 | 110.4 | 27.6 |

NOTE: Cold corresponds to <10°C.
Warm corresponds to >10°C.

Since most workers were considered to work primarily during daylight hours, the average daytime temperature (average temperature between 8:00 A.M. and 5:00 P.M.) was estimated for each month and each state. Numerical integration was used to calculate the average 24 hour temperature (AT), the average daytime temperature (ADT), and the maximum temperature (MT), based on a typical daily temperature cycle (Figure 1). These three quantities were used to calculate a temperature factor (TF) defined by the following equation:

$$ADT = AT + TF * (MT - AT).$$

Separate temperature factors were estimated for clear and cloudy days:

$TF_{clr} = 0.77$ (clear)

$TF_{cdy} = 0.51$ (cloudy)

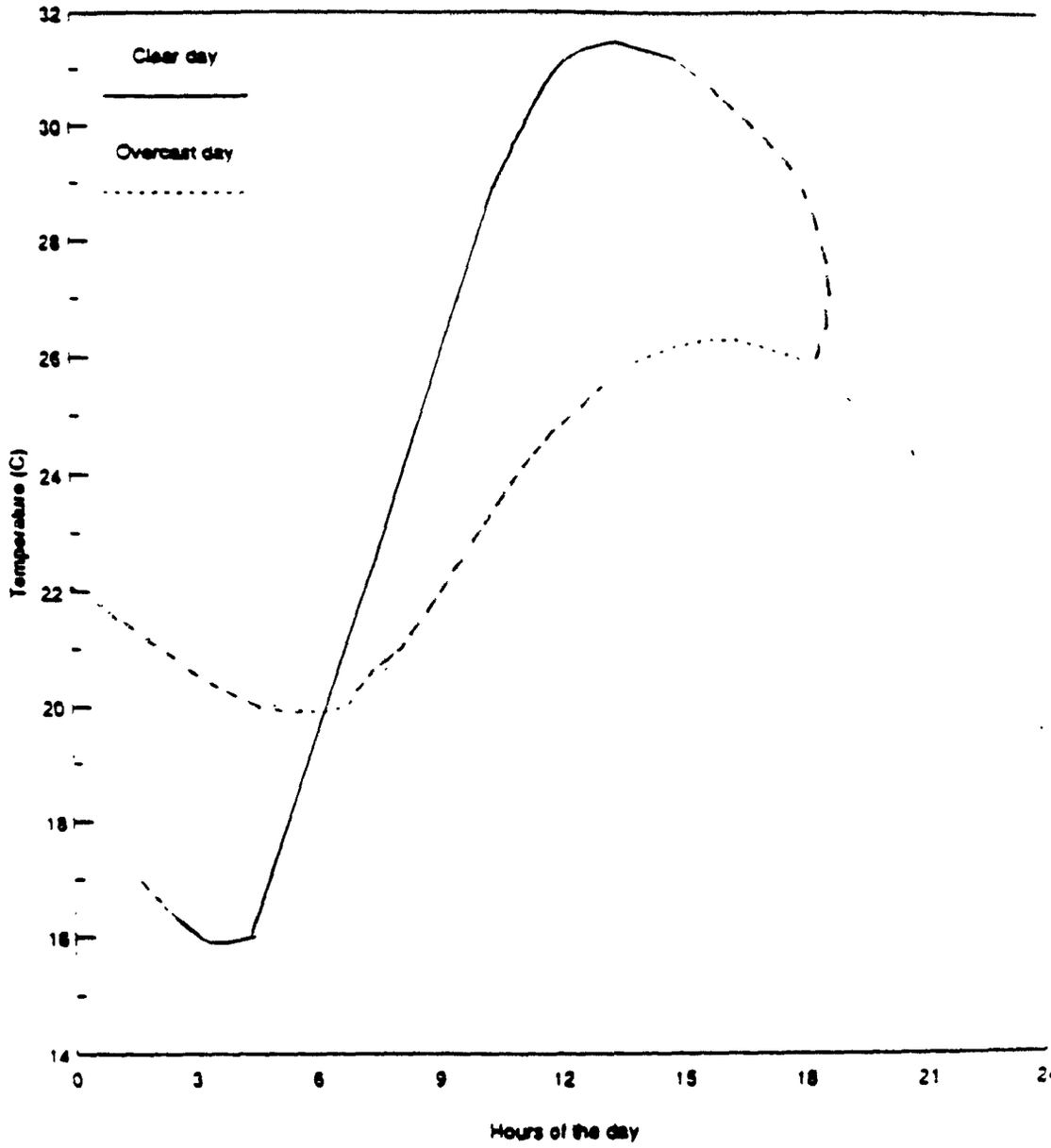
These temperature factors were then used to estimate the average daytime temperature for each month from the NOAA (1978) data as follows:

$$[\text{AVERAGE DAYTIME MONTHLY TEMP}] = [\text{AVE. MONTHLY TEMP.}] + ([\text{AVE. MAX. DAILY TEMP. FOR MONTH}] - [\text{AVE. MONTHLY TEMP.}])) * \left\{TF_{clr} * \frac{[\text{FRACTION CLEAR DAYS}] + [\text{FRACTION PARTLY CLOUDY DAYS}]}{2} + TF_{cdy} * \frac{[\text{FRACTION CLOUDY DAYS}] + [\text{FRACTION PARTLY CLOUDY DAYS}]}{2}\right\}$$

Average temperatures for the years 1941 through 1970 were used in these calculations. If [AVERAGE DAYTIME TEMP] was above 10°C for a particular month, that month was designated as a warm month; otherwise it was designated as a cold month. These calculations were performed for each state. A summary of these calculations is provided in Table 4.

To calculate a worker's "climate-adjusted" exposure to a particular marker of diesel particulate for a given year, a weighted average of the "warm" and "cold" exposures (listed in Table 3) corresponding to his job category for that year was calculated, with the weights being the proportion of warm or cold months listed in Table 4. These weighted exposures were assumed to apply from 1959 onward.

Figure 1




The daily temperature cycle on a clear day versus a cloudy day
Cloud cover will depress the daily maximum while raising the daily minimum temperature

Source: Oliver (1987).

No address was available for a number of the smaller railroads. Since no climate-adjusted exposures could be developed for workers in these railroads, analyses involving climate-adjusted exposures omitted these workers. There were 897 workers in this category (1.6% of cohort).

**Assigning Exposures in Unsampled Job Categories.** The 13 worker categories in Table 3 that were assigned exposures correspond to the 13 categories of job codes for 1959 that were sampled in the Garshick *et al.* (1988) study, and which are listed in Table 2. The lower part of Table 2 contains a list of job codes that were assigned in subsequent years, but were not sampled in 1959. An unsampled job was assigned the same exposure as a sampled job that appeared to involve similar exposures. "Other train riders" were assigned to the same exposure category as conductors, brakemen, and hostlers, and "carmen" were assigned to the same category as clerks. Persons with an unknown job in a given year (ICC code 000) were assigned to the same exposure category as the previous year. These assignments should have a very limited effect because the unsampled job codes corresponded to only a small fraction of the total person-years of follow-up (other train riders, 0.2%; carmen, 0.5%; unknown job, 0.5%).

**Estimation of Exposures Prior to 1959.** Since job codes are not available prior to 1959, workers were assumed to have had the same job from the beginning of employment to 1959 as they had in 1959. Since information is not available concerning which workers worked near diesel equipment during the years prior to 1959 in which both diesel and steam equipment were used, it was assumed that exposures in each job category increased linearly with time from 1945 through 1959. This linear increase parallels the overall rate at which the locomotive fleet became dieselized (AAR, 1989).

The first year of employment by a railroad was specified in the data base provided by the RRB for only 2,908 workers (2%), all of which began work in 1947 or later. However, the total months of railroad service was available for each worker, and the starting date of work was estimated based on total service months and last year of work.

Table 4
Number of Months per Year the Average
Temperature is Greater than 10°C by State

| STATE | MONTHS | STATE | MONTHS |
|---|---|---|---|
| Alabama | 12 | Montana | 6 |
| Alaska | 5 | Nebraska | 6 |
| Arizona | 7 | Nevada | 6 |
| Arkansas | 9 | New Hampshire | 6 |
| California | 12 | New Jersey | 8 |
| Colorado | 7 | New Mexico | 9 |
| Connecticut | 6 | New York | 6 |
| Delaware | 8 | North Carolina | 9 |
| Florida | 12 | North Dakota | 6 |
| Georgia | 12 | Ohio | 7 |
| Hawaii | 12 | Oklahoma | 9 |
| Idaho | 7 | Oregon | 8 |
| Illinois | 7 | Pennsylvania | 7 |
| Indiana | 7 | Rhode Island | 6 |
| Iowa | 6 | South Carolina | 12 |
| Kansas | 9 | South Dakota | 6 |
| Kentucky | 8 | Tennessee | 9 |
| Louisiana | 12 | Texas | 12 |
| Maine | 6 | Utah | 7 |
| Maryland | 8 | Vermont | 6 |
| Massachusetts | 6 | Virginia | 9 |
| Michigan | 6 | Washington | 7 |
| Minnesota | 6 | West Virginia | 9 |
| Mississippi | 12 | Wisconsin | 6 |
| Missouri | 8 | Wyoming | 6 |

This approach will overestimate the start date for workers whose employment was not continuous. To give some idea of the error introduced by this approximation, the estimated first year of work was compared to the actual year for the 2,908 workers whose starting year was known. In this sample, the estimated starting year was correct for 2,336 workers (80%), one year too high for 463 workers (20%), two years too high for 104 workers (3.6%) and three years too high for five workers (0.2%). Thus, in this sample, at least, the method of estimating the starting year did not introduce gross errors. It should be noted that this method will provide an accurate estimate of total years of exposure prior to 1959, although some of the exposures may have occurred somewhat earlier than estimated.

## METHODS OF ANALYSIS

### Methods Involving Poisson Regression

The principal analytic method used to evaluate the relationship between exposure variables and lung cancer incidence was Poisson regression. [See BEIR IV (1988) and BEIR V (1990) and references contained therein for a detailed discussion of the method.] In this approach, each explanatory variable (e.g., age, calendar year, exposure measure, etc.) is categorized by dividing its range into subintervals. This categorization is used to cross-tabulate the data into cells, with each cell containing data corresponding to a particular category (subinterval) of each explanatory variable. Each cell will thus contain data corresponding to similar values of each explanatory variable. Data tabulated into cells include the person-years of follow-up and the number of cases. The average value of one or more of the explanatory variables may also be calculated for each cell, with the average being weighted by the number of person-years corresponding to the cell. Since the values of some explanatory variables for a single individual in the cohort may vary with time (e.g., cumulative exposure), the experience of a single cohort member may not be summarized in a single cell but rather contribute to a number of cells.

The number of cases occurring in each cell is assumed to have a Poisson distribution with the expected number of cases being the product of the number of person-years in that cell and the risk per person-year predicted by a statistical model. The data are then fit by the process of maximum likelihood (Cox and Lindley, 1974) and

hypothesis tests and confidence intervals are computed based on a large sample maximum likelihood theory.

Several explanatory variables were selected that were considered to be potentially important predictors of lung cancer risk in this cohort. Non-exposure variables used in the analysis included age, calendar year, and job category in 1959.

In most of the analyses, age was divided into five 10-year intervals: 40-49, 50-59, 60-69, 70-79, and 80+. In some of the analyses, age was divided into ten 5-year intervals beginning with 40-45. A few of the analyses were conducted twice using both divisions of age in order to evaluate the effect of subdividing age in different ways.

Calendar year was categorized into either seven intervals (1959-1965, 1966-1968, 1969-1971, 1972-1973, 1974-1977, 1978-1979, and 1980) or 22 intervals (each interval corresponding to one calendar year). Job code in 1959 was categorized mainly according to five major divisions: clerk; signalman; engineer and firer; conductor, brakeman, and hostler; and shop worker. However, exposures were estimated separately for each of the 13 job groups listed in Tables 2 and 3.

Four different exposure variables were created for each exposure measure. These were: cumulative exposure omitting most recent three years (current year and two previous years); cumulative exposure in past four through eight years (omitting most recent three years); cumulative exposure in past nine through 18 years; and cumulative exposure more than 18 years in the past. The first of these exposure variables was categorized into six subintervals and the remaining three generally into four subintervals. Class boundaries were set so that roughly equal numbers of person-years were in each subinterval. These different exposure variables were used in order to investigate different potential latencies for lung cancer induced by DE; i.e., to determine whether exposures during a particular period in the past were more highly correlated with lung cancer risk than exposures during other periods. If this were the case, then including exposures during other periods could mask an effect of diesel exposure.

These exposure variables were created for each of the five markers of diesel exposure considered (ARP, climate-adjusted ARP, climate-adjusted AEM, climate-adjusted RSP, and climate-adjusted TEX).

There is some evidence from animal studies that the clearance of respirable particles from the lung is capacity limited (Morrow, 1988). This would suggest that more intense exposures may be more dangerous per amount inhaled than less intense exposures. To explore this hypothesis, a maximum exposure variable was created for each of the five markers of diesel exposure, defined as the maximum one year exposure occurring more than three years in the past. If more intense exposures are the most dangerous, then lung cancer risk could correlate more closely with maximum exposure than cumulative exposure.

Several types of statistical models were applied to the cross-tabulated data. First, the risk of lung cancer was modelled as

$$risk_{ijk} = a_i \, y_j \, e_k,$$

where $risk_{ijk}$ is the risk per person-year in the cell corresponding to the ith age category, jth calendar year category, and the kth exposure category, and $a_i$, $y_j$, and $e_k$ are estimated parameters. The dose response was indicated by plotting the exposure parameters $e_k$, along with 90% confidence intervals, versus the average exposure in the kth exposure subinterval.

Second, the risk of lung cancer was modeled as

$$risk_{ij}(e) = a_i \, y_j \, [1 + \beta \, e],$$

where $risk_{ij}(e)$ is the risk in the ith age group and jth calendar year category from an exposure e. The exposure used in applying the model was the average exposure in a cell. In this model a single dose parameter, $\beta$, is estimated rather than a different parameter for each dose category. The latter approach gives a more complete description of the dose response, whereas the former approach summarizes the dose response in a single variable, $\beta$. A positive value of $\beta$ corresponds to a positive relation between exposure and lung cancer and a negative value for $\beta$ corresponds to an inverse relationship (higher exposures being associated with lower risks).

Both of the above models are multiplicative models, i.e., they assume that the effect of diesel exposure is to multiply the background risk of lung cancer. A few analyses were based on the additive model

$$risk_{i,j,k} = a_i\, y_j + a'_i e_k.$$

In this model diesel exposure is assumed to increase the background risk of lung cancer in an age-dependent additive manner.

In addition to being applied to the complete cohort, these models were also applied to various subsets of the cohort defined by job in 1959, or by age in 1959.

In some of the analyses, exposure was a dichotomous variable (yes/no). As in Garshick *et al.* (1988), clerks and signalmen comprised the unexposed group in these analyses.

**Methods Using Comparisons with U.S. Rates**

One analysis was conducted to compare the cancer and overall death rates in this cohort with comparable rates from the general U.S. population. Observed numbers of lung cancer deaths in various categories were compared with expected numbers of deaths and based on age- and calendar year-specific mortality rates for U.S. white males.

**Methods Using Partial Likelihoods**

An analysis based on a proportional hazard model implemented using the partial likelihood method of Cox (1972) was used to model the dependence of relative risk of lung cancer upon elapsed time from beginning of exposure (1959) to five years prior to death or retirement. In contrast to the application used by Garshick *et al.* (1988), the risk sets were defined by age rather than by calendar year. Twenty-two category variables, one for each year, were used to control for calendar year. Workers classified as clerks or signalmen in 1959 were considered to be unexposed (i.e., these workers were assigned an elapsed time of zero).

The software package EPICURE (Preston *et al.*, 1990) was used to implement all of the analyses in this report.

## RESULTS

### Comparisons of Exposed to Unexposed Workers Independent of Exposure Level

Table 5 contains the observed and expected number of cancers (expected calculated from age- and calendar year-specific death rates for U.S. white males), and the associated relative risks, by age in 1959 and by exposed and unexposed workers. The last column of Table 5 contains the ratios of relative risks for exposed workers to those for unexposed workers. This column confirms the finding by Garshick *et al.* (1988) of higher risks in exposed workers relative to unexposed, particularly among the workers who were younger in 1959. The ratios of relative risks in Table 5 are almost identical to the relative risks appearing in Table 5.

Table 5
Lung Cancer Deaths in Garshick et al. (1988)

| | Unexposed Workers | | Exposed Workers | | |
|---|---|---|---|---|---|
| Age in 1959 | Observed Deaths | Relative Risk | Observed Deaths | Relative Risk | RR Exposed/ RR Unexposed |
| 40-44 | 67 | 0.63 | 291 | 0.90 | 1.43 |
| 45-49 | 69 | 0.69 | 309 | 0.93 | 1.34 |
| 50-54 | 90 | 0.87 | 280 | 0.97 | 1.12 |
| 55-59 | 92 | 1.03 | 285 | 1.22 | 1.18 |
| 60-64 | 64 | 1.12 | 147 | 1.11 | 0.99 |

As discussed by Garshick *et al.* (1988), since the younger workers in 1959 were the ones with the potentially longest exposures, the trend indicated by the last column of Table 5 is consistent with an effect of diesel exposure upon lung cancer. However, Table 5 reveals some features of the lung cancer rates that were not apparent from the internal analysis conducted by Garshick *et al.* (1988). Within each group of workers (unexposed and exposed), the risk relative to U.S. rates increases with age in 1959. However, this increase is greater among unexposed than exposed workers, which accounts for a decreasing relative risk with age in 1959 in internal comparisons of exposed and unexposed. Consequently, using U.S. rates as a comparison population, relative risk increases, rather than decreases, with age in both the exposed and unexposed groups.

In order to explore the reason for this behavior, the pattern of both lung cancer deaths and total deaths by calendar year were studied. Figure 2 displays graphs of the percent of persons, by year, alive at the beginning of the year and who die of any cause during the year. The figure contains a separate graph for each five-year age group beginning with ages 40-44. The graphs for most age groups do not extend across all years because there were no cohort members in some age groups in certain years. (E.g., since everyone in the cohort was at least 40 years old in 1959, by 1964 there were no longer any cohort members aged 40-44.)

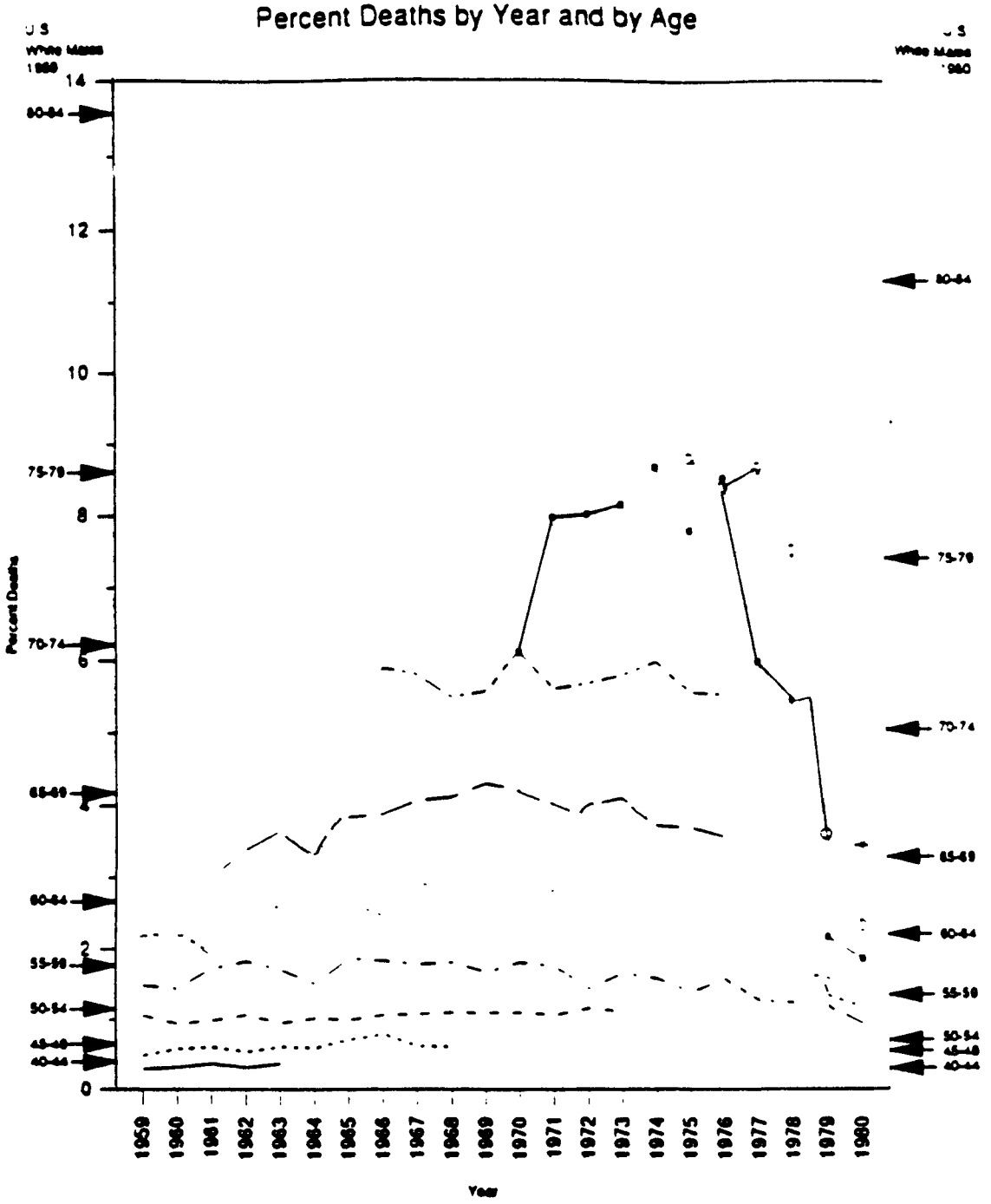
Up through about 1976, the age-specific death rates remained roughly constant, which is consistent with the pattern of death rates for the U.S. during this period. However, beginning in about 1976 the death rates begin to decrease precipitously with time. By 1980 the death rates in men aged 80-85 was similar to that of men aged 60-65 between 1959 and 1976. Similarly, in 1980, the death rates for men aged 65-70 were similar to those aged 50-55 between 1959 and 1976. Although there were too few deaths from lung cancer to reliably graph such trends for each five-year age interval, the total death rate for lung cancer decreased after 1976 in a manner that paralleled the decrease in total deaths.

Figure 2 also indicates the death rates for U.S. white males for the years 1959 and 1980. Although the rates for 1980 are somewhat lower than those for 1959, rates for these two years are similar and both sets of rates are comparable to the death rates in the cohort between 1959 and about 1976. However, death rates in the cohort begin to fall below U.S. rates in years subsequent to 1976. By 1980, the rates for the cohort were below U.S. rates by factors ranging from two (for ages 60-65) to almost six (for ages greater than 85). Thus, not only did death rates in the cohort decrease after 1976, they were comparable to U.S. rates prior to 1976 and decreased to values considerably below U.S. rates in subsequent years.

Consideration was given to the reason for the observed drop-off in mortality in this cohort. It is highly unlikely that it is an effect of diesel exposure. The possibility that it was a manifestation of the health worker effect was also considered. However, to the extent that such an effect is associated with cancer response, it should have been more apparent in the early years of follow-up rather than in later years.

Figure 2

Percent Deaths by Year and by Age




| | | | | |
|---|---|---|---|---|
| 40 - 44 | 45 - 49 | 50 - 54 | 55 - 59 | 60 - 64 |
| 65 - 69 | 70 - 74 | 75 - 79 | 80 - 84 | 85 + |

The only plausible explanation that has been proposed for the drop-off in mortality is that it is due to a lack of follow-up in the cohort; deaths must have occurred that were not included in the file provided by the RRB.[1] The RRB was the only source of death ascertainment in the Garshick et al. (1988) study, so there was no verification of death ascertainment through an independent source.

Since there is evidence that not all deaths were recorded from about 1977 onward, analyses were conducted to determine if the patterns seen in Table 5 still appear if follow-up was stopped at the end of 1976. Table 6 shows the results of a Poisson

Table 6
Relative Risks of Lung Cancer Death in Exposed Versus Unexposed Workers for Different Follow-up Periods

| Age in 1959 | Follow-up through 1980 Exposed vs. Unexposed (95% CI) | Follow-up through 1976 Exposed vs. Unexposed (95% CI) |
|---|---|---|
| 40-44 | 1.49 (1.1 - 1.9) | 1.64 (1.2 - 2.3) |
| 45-49 | 1.31 (1.0 - 1.7) | 1.25 (0.93 - 1.7) |
| 50-54 | 1.12 (0.88 - 1.4) | 1.20 (0.93 - 1.6) |
| 55-59 | 1.17 (0.92 - 1.5) | 1.12 (0.88 - 1.4) |
| 60-64 | 0.96 (0.74 - 1.3) | 1.17 (0.70 - 1.3) |

regression analysis based on the complete data set, and secondly, based upon a truncated data set in which follow-up is assumed to end on December 31, 1976. When follow-up continues through 1980, the results of the Poisson regression agree closely with both

[1] It has recently been reported (Garshick, 1991) that a new tape provided by the RRB includes follow-up of the cohort for additional years, as well as an update of the follow-up through 1980. For 1980, about 25% of the deaths on the updated tape were not on the earlier tape. Some additional deaths are also on the new tape for 1979, but the percentage is much smaller. However, Figure 2 suggests that the lack of follow-up was more severe than this.

those in Table 5 obtained by comparison with U.S. rates, and those reported in Garshick *et al.* (1988) Table 5 obtained using partial likelihood methods. When follow-up is stopped at the end of 1976, the results do not change materially. There is still a trend toward higher risks in exposed workers relative to unexposed workers who were younger in 1959, and the excess lung cancer among exposed workers is statistically significant in men aged 40-44 in 1959. Moreover, the relative risk is higher among workers aged 40-44 in 1959 when follow-up stops in 1976 than when it continues through 1980. This finding indicates that the excess relative risk among exposed workers found by Garshick *et al.* (1988) holds even if data after 1976 are omitted from the analysis.

Table 7 indicates the relative risk in exposed versus nonexposed for different groups of exposed workers. Among engineers and firers, the relative risk is significantly elevated among those who were 40-44 in 1959 and nearly so among those who were 45-49 in 1959. The data for engineers and firers also exhibit the monotone relationship between relative risk and age in 1959 that is seen in the complete cohort of exposed workers (Table 6). This group also exhibits an almost significant deficit of lung cancer relative to unexposed workers among those 60-64 years of age in 1959.

Table 7
Relative Risks of Lung Cancer in Exposed
Versus Unexposed for Different Exposed Groups

| Age in 1959 | Engineers and Firers | Brakemen, Conductors and Hostlers | Shop Workers |
|---|---|---|---|
| 40-44 | 1.71 (1.2, 2.4) | 1.58 (1.2, 2.1) | 1.13 (0.79, 1.6) |
| 45-49 | 1.34 (0.97, 1.8) | 1.29 (0.97, 1.7) | 1.30 (0.94, 1.8) |
| 50-54 | 1.44 (1.1, 1.9) | 1.08 (0.81, 1.4) | 0.92 (0.68, 1.2) |
| 55-59 | 1.04 (0.76, 1.43) | 1.28 (0.97, 1.7) | 1.15 (0.87, 1.5) |
| 60-64 | 0.70 (0.45, 1.1) | 1.26 (0.88, 1.8) | 0.92 (0.64, 1.4) |

Brakemen, conductors and hostlers exhibit a relative risk greater than unity in each age category, and the excess is statistically significant among workers 40-44 years of

age in 1959. However, the pattern of higher relative risks among workers who were younger in 1959 is not present; rather, relative risks are higher in the youngest and oldest age categories and lower in the middle age categories.

Among shop workers, the relative risk is not significantly elevated in any age group. Furthermore, there is no statistically significant evidence of an increased risk among shop workers, either overall ($p = 0.13$ based on a one-sided test of significance of a single relative risk applied across all age groups) or in any five-year age group based on age in 1959. There is also no discernable pattern of variation of relative risk with age in 1959.

**Analyses of Cumulative Years of Exposure or Elapsed Years Since Beginning of Exposure**

The partial likelihood analysis of relative risk with elapsed time from 1959 until four years prior to death or retirement found a statistically significant excess relative risk among exposed workers in the two lowest categories of elapsed time but not in the two highest, and the excess did not increase monotonically with elapsed time. The relative risk of lung cancer with 1-4 years of elapsed time was 1.25 (95% CI = 1.04, 1.51), for 5-9 elapsed years was 1.34 (95% CI = 1.15, 1.56), for 10-13 elapsed years was 1.15 (95% CI = 0.97, 1.36), and for 15-17 years was 1.33 (95% CI = 0.98, 1.82). A corresponding analysis based on Poisson regression gave almost identical results. The relative risk of lung cancer with 1-4 years of elapsed time was 1.26 (95% CI = 1.04, 1.52), for 5-9 years was 1.34 (95% CI = 1.14, 1.56), for 10-13 elapsed years was 1.14 (95% CI = 0.96, 1.35), and for 15-17 elapsed years was 1.32 (95% CI = 0.97, 1.79). In this analysis, calendar year was controlled for using an indicator variable for each year, and age was controlled for using an indicator variable for each five-year age interval.

A Poisson regression analysis found an inverse relationship between cumulative years of exposure lagged five years and excess relative risk. The relative risk of lung cancer for 1-4 cumulative years of exposure was 1.38 (95% CI = 1.17, 1.62), for 5-9 years of cumulative exposure was 1.28 (95% CI = 1.10, 1.49), for 10-14 years was 1.10 (95% CI = 0.92, 1.3), and for 15-17 years was 1.06 (95% CI = 0.75, 1.50). In this analysis, persons classified as clerks, signalmen or carmen during a year were considered to be unexposed during that year. A person with a missing job code was assigned the job code for the previous year. Cumulative years of exposure were calculated by summing the

months of exposure by year and dividing by twelve. Calendar year was controlled for using an indicator variable for each year and age was controlled for by using an indicator variable for each five-year age interval. Exposures during the current year and the most recent four years were ignored.

**Dose Response Analyses**

Table 8 summarizes the results of 50 dose response analyses in which exposure was treated as a continuous variable. An estimated dose coefficient and associated standard error is provided for each analysis. A positive dose coefficient indicates a positive relationship between exposure and lung cancer risk, whereas a negative coefficient indicates an inverse relationship. Although the model being applied assumes a linear dose response, a positive dose response should result in a positive coefficient even if the dose response is non-linear.

All but two of the 50 analyses produced a negative dose coefficient.[2] The two exceptions involved signalmen, who are considered to have had only limited exposure. Many of the negative values were statistically significant (absolute value of the dose coefficient greater than twice the standard error).

Figures 3 through 12 summarize the results of selected dose response analyses in which independent dose coefficients are estimated for each exposure interval. In each figure, the risk in the lowest exposure group has been normalized at 1, and the risk in each exposure group relative to that in the lowest exposure group is plotted at the average exposure in that group, along with the 90% confidence interval on the relative risk.

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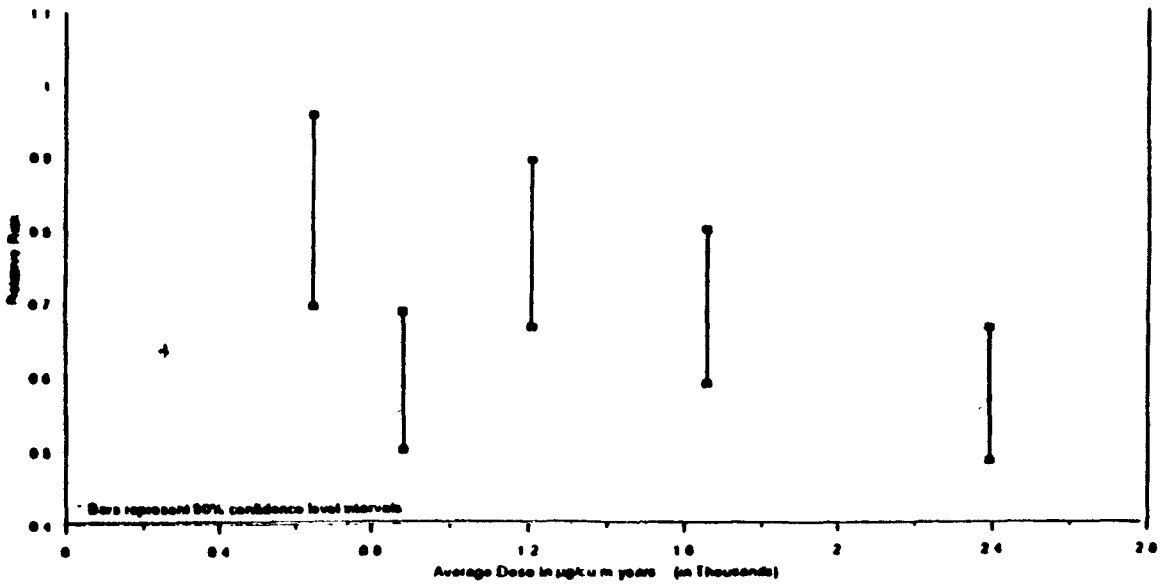
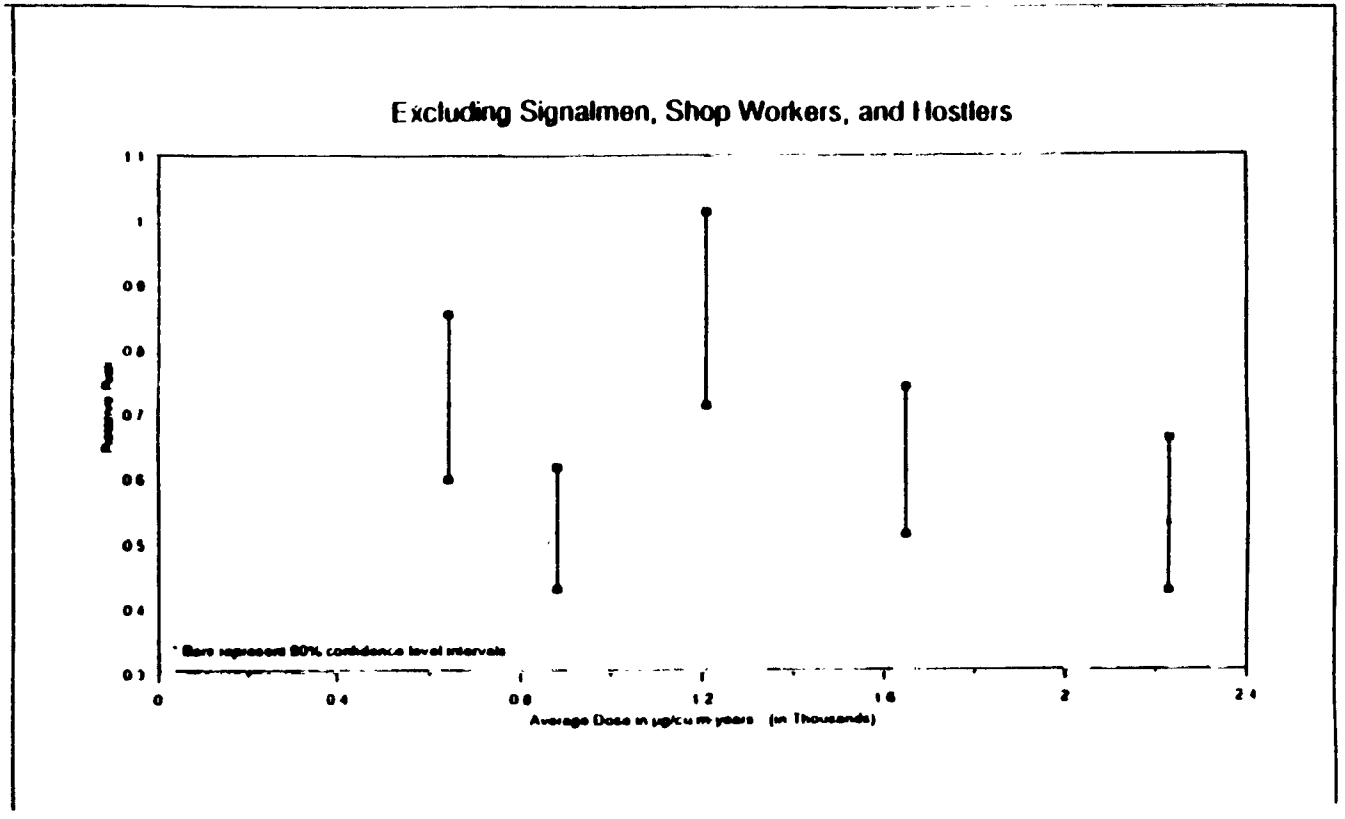
[2]A number of these analyses produced negative coefficients so large that negative probabilities were estimated for a few cells. To allow the program to converge, a few cells were deleted from the analysis whenever this occurred. The deleted cells contained, at most, only 2.4% of the total person-years included in an analysis.

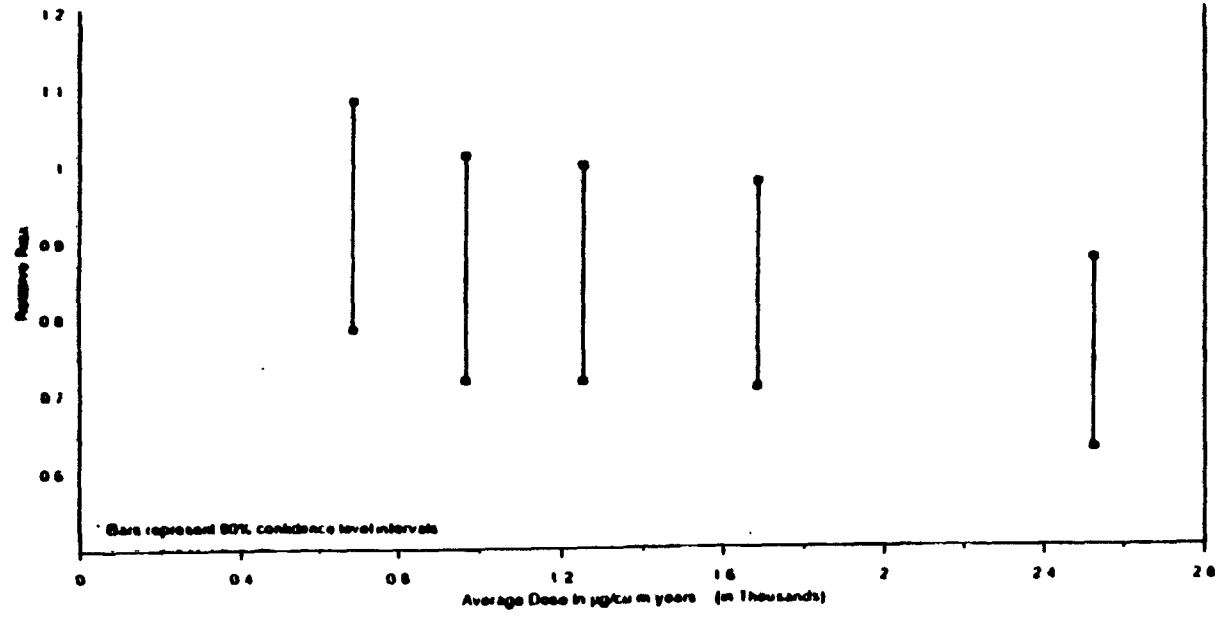
# Figure 3
# Lung Cancer Risk Versus Unadjusted ARP



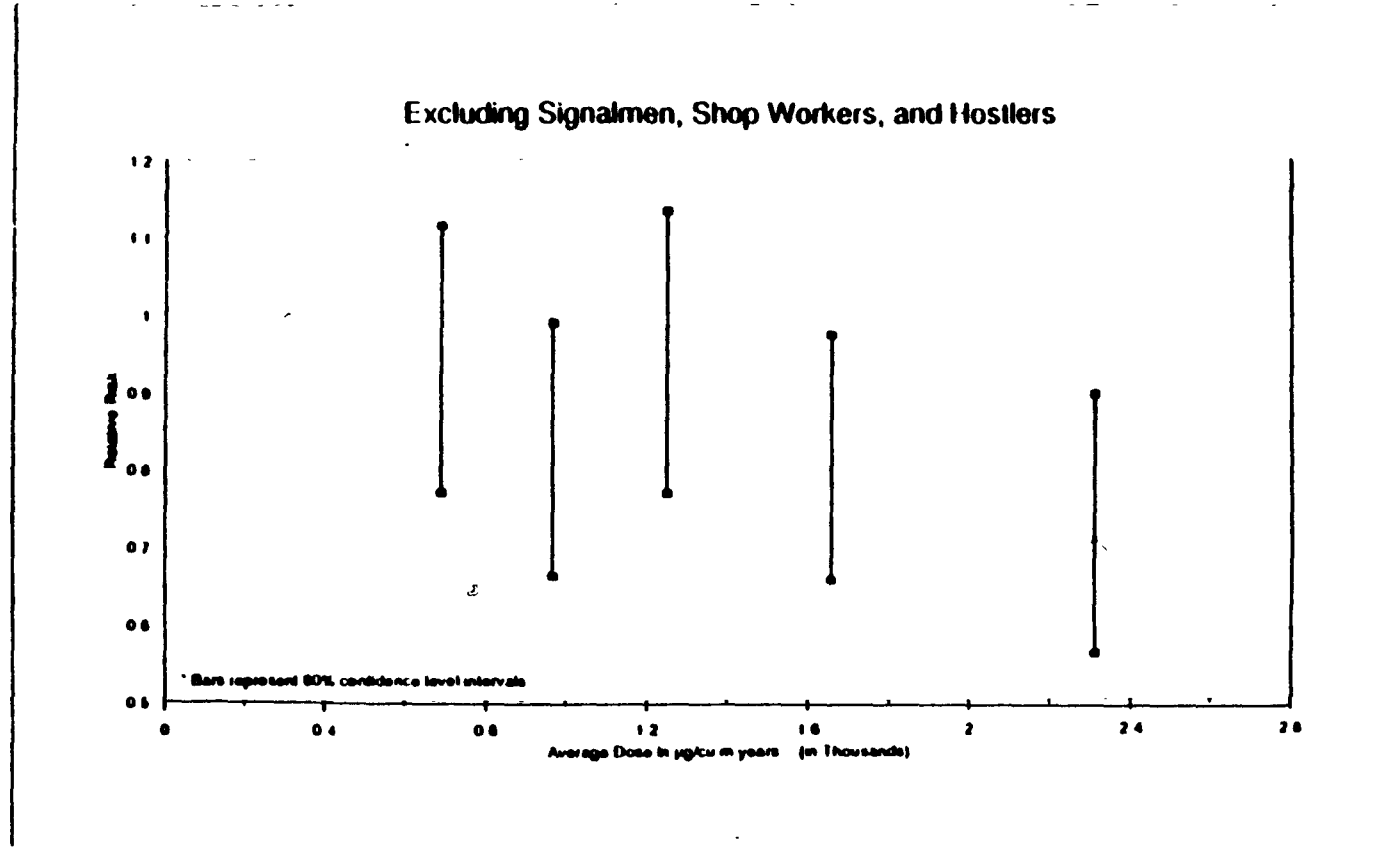
# Figure 4
# Lung Cancer Versus Unadjusted ARP



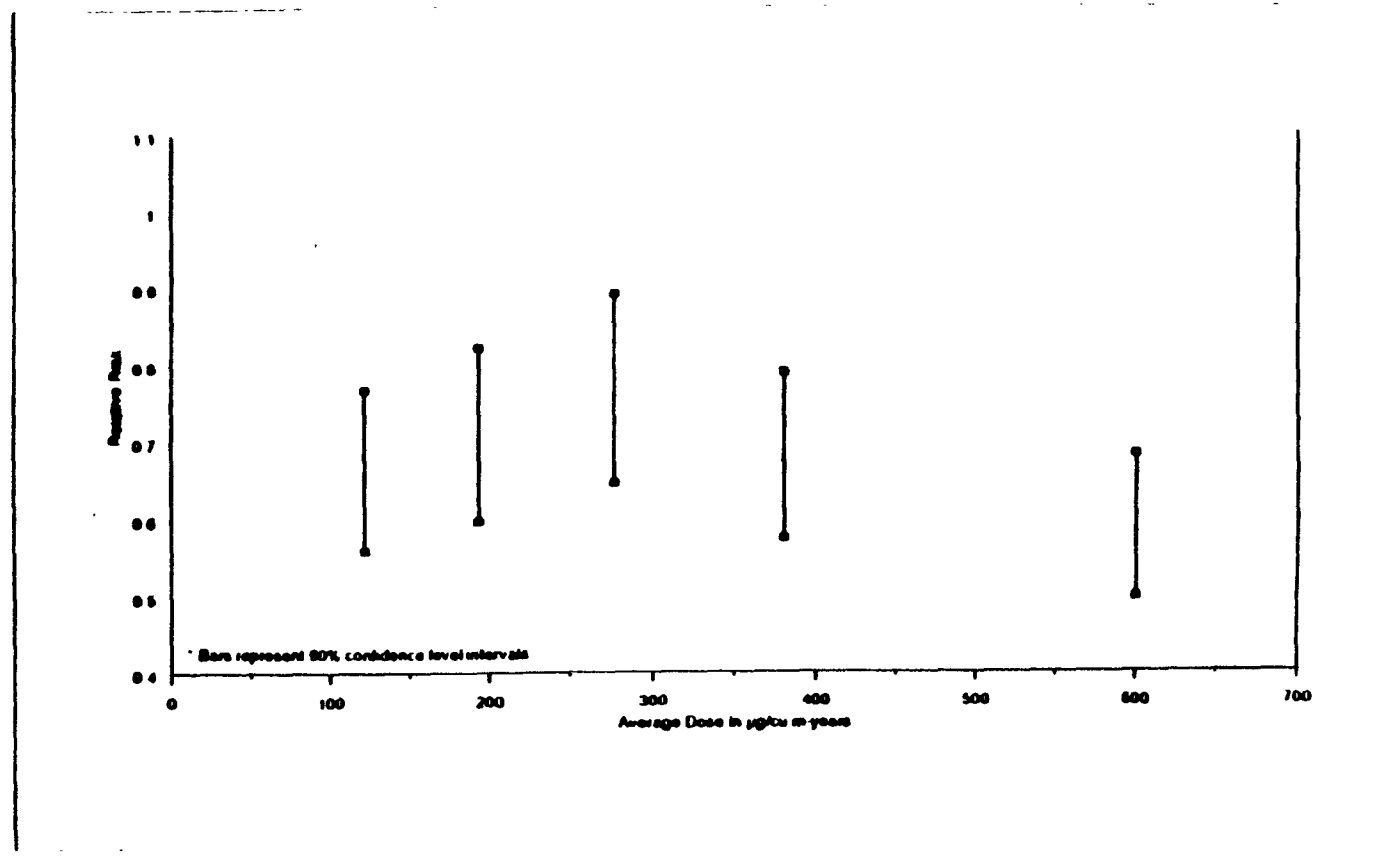
# Figure 5
# _ung Cancer Risk Versus Climate Adjusted ARP



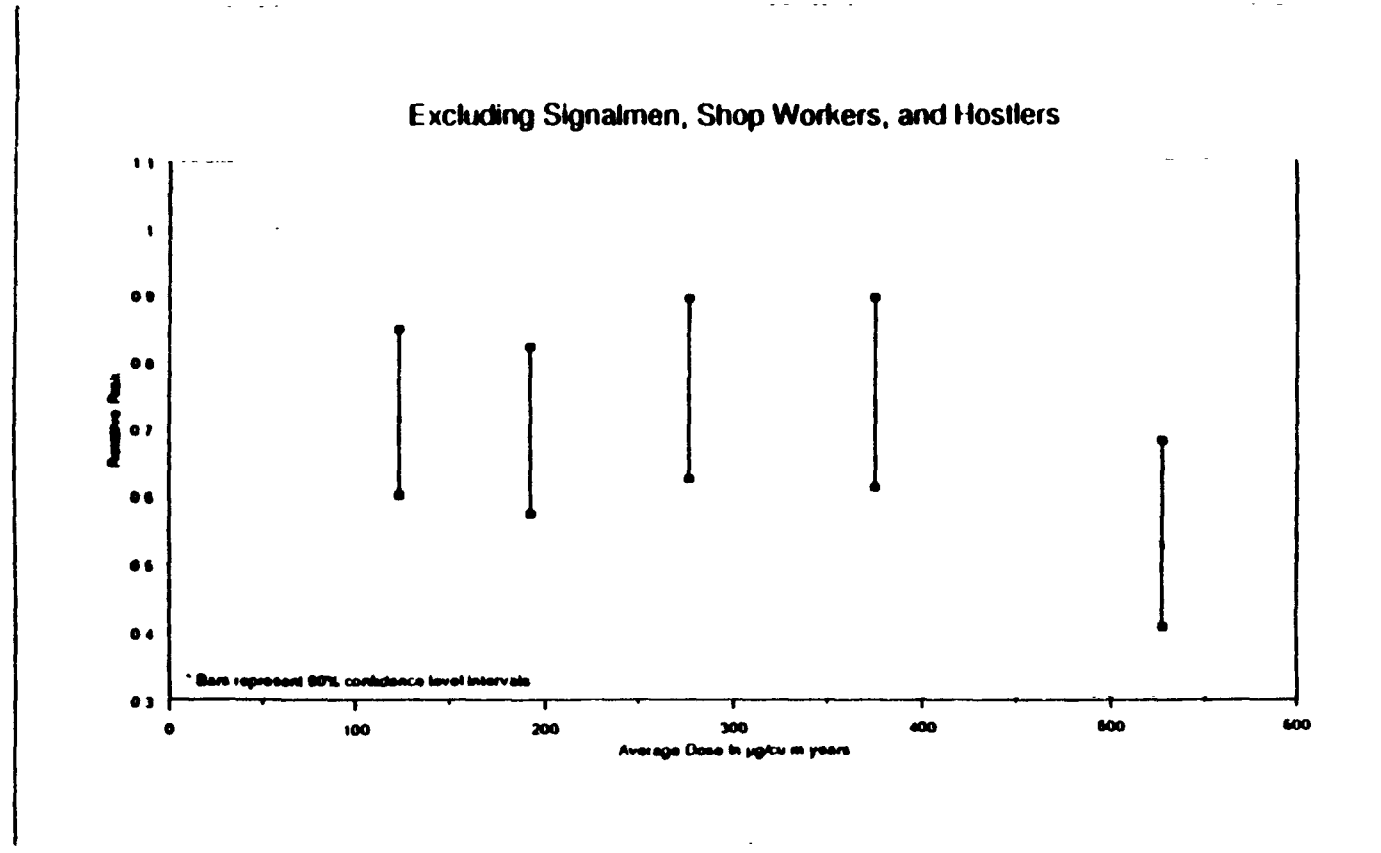
# Figure 6
# Lung Cancer Risk Versus Climate Adjusted ARP



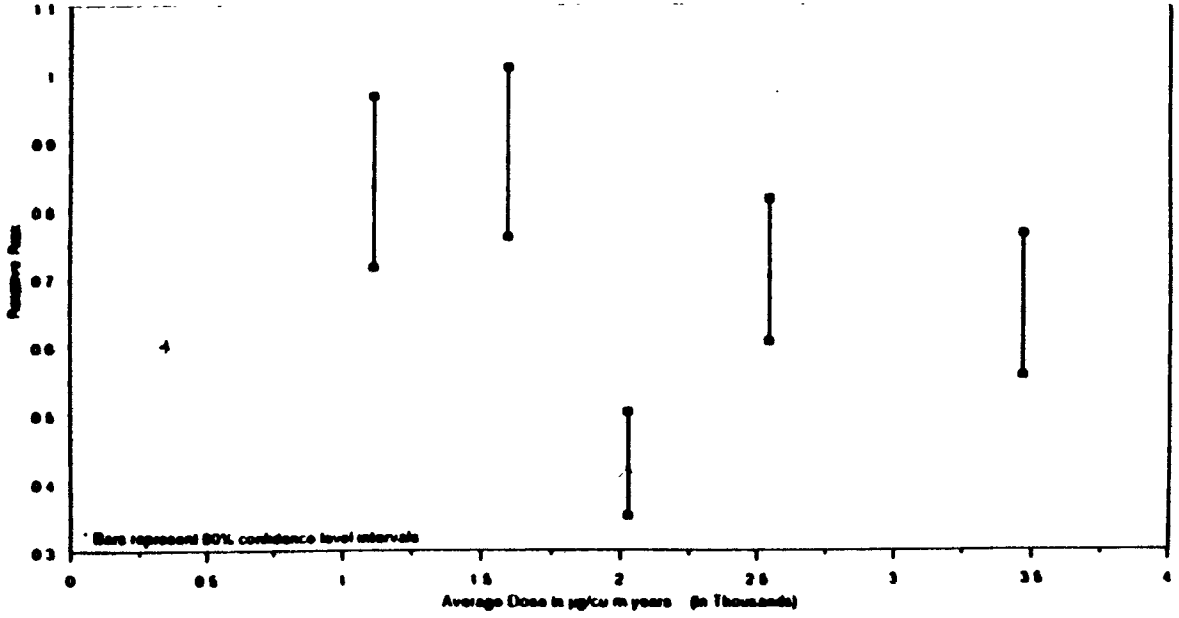
# Figure 7
# Lung Cancer Risk Versus Climate Adjusted AEM



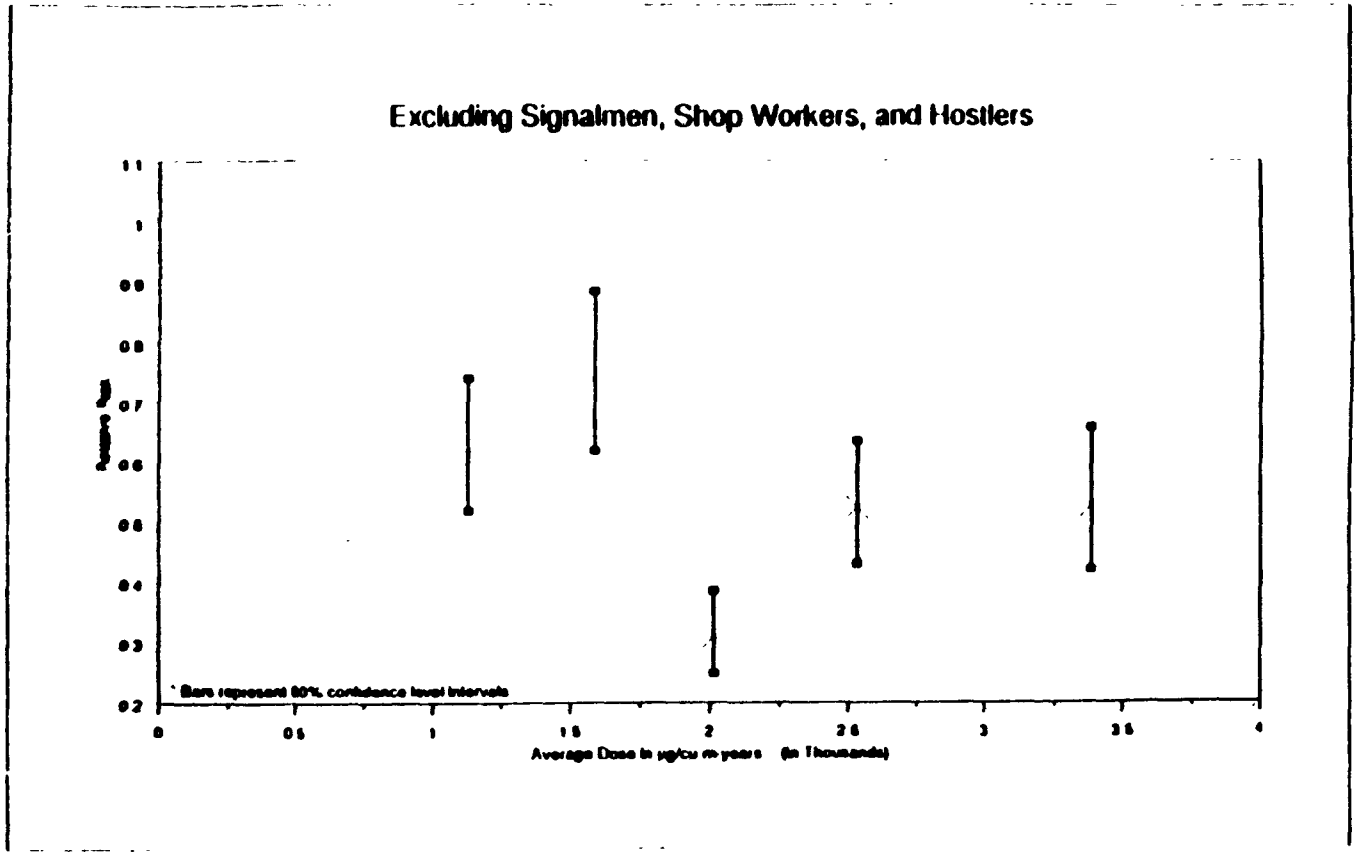
# Figure 8
# Lung Cancer Risk Versus Climate Adjusted AEM



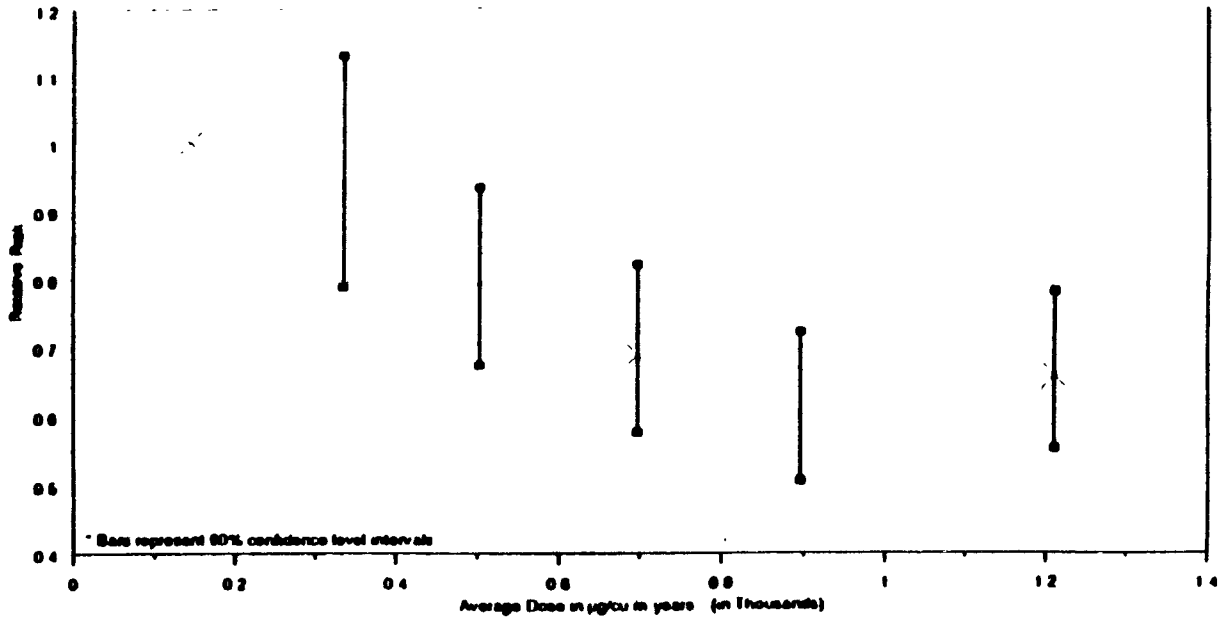
# Figure 9
# Lung Cancer Risk Versus Climate Adjusted RSP



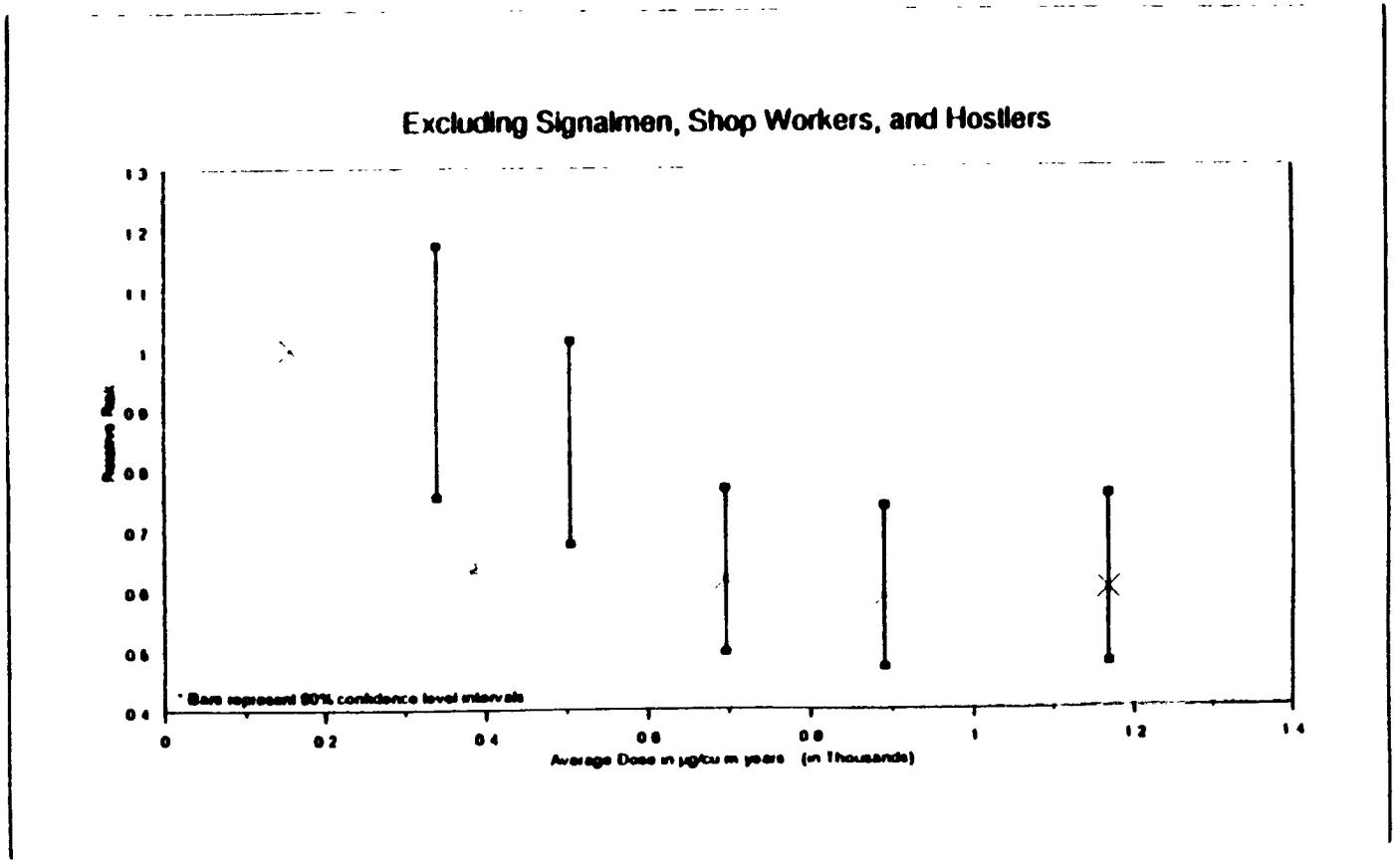
# Figure 10
# Lung Cancer Risk Versus Climate Adjusted RSP



# Figure 11
# Lung Cancer Risk Versus Climate Adjusted TEX



# Figure 12
# Lung Cancer Risk Versus Climate Adjusted TEX



Each of these figures shows the dose response for cumulative exposure four or more years in the past corresponding to one of the five markers for diesel exposure. Figures are presented for both the entire cohort and for the subcohort defined by omitting data on signalmen, hostlers, and shop workers. These jobs were omitted in order to emphasize jobs for which exposure estimates are considered to be the mostreliable. A number of similar plots using other exposure variables are shown in Appendix E. None of these plots (including those in Appendix E) indicate a positive relationship between a marker for diesel exposure and risk of lung cancer. Indeed, as a whole, they tend to indicate a negative relationship.

Since the highest relative risk in Table 5 is for persons aged 40-45 in 1959, the analyses in Table 8 and Figures 3 through 12 were repeated for cumulative exposure to unadjusted ARP more than three years in the past for the complete cohort, engineers/firers, conductor/brakemen, and shop workers, but restricted to persons aged 40-45 in 1959. These analyses did not indicate a relationship between exposure and lung cancer. The dose response slope was negative for the complete cohort, engineer/firers, and conductors/brakemen. Although the slope was slightly positive for shop workers, it was not statistically significantly different from zero.

Table 9 presents results from applying the Poisson regression model in which cumulative exposure to ARP (unadjusted for climate) more than three years in the past was entered categorically in an additive fashion. As with the analyses in which exposure was entered in a multiplicative fashion (Figures 3 through 7), this analysis suggests a negative relationship between cumulative exposure to DE and lung cancer. Similar results were obtained when this model was applied to the other four markers of diesel exposure.

Maximum one-year exposure occurring more than three years in the past was not positively correlated with lung cancer relative risk for any of the five markers of diesel exposure considered. In fact, the dose response slope was negative for each of the five markers.

| Table 9<br>Relation Between Lung Cancer Mortality and Exposure to ARP Using an Additive Risk Model | | |
|---|---|---|
| Average Exposure to ARP ($\mu g/m^3$-years) | Increase in Lung Cancer Risk Relative to Lowest Exposure Group | 95% Confidence Interval |
| 320 | 0.0 | --- |
| 650 | -0.18 | (-0.44, -0.086) |
| 880 | -0.95 | (-1.3, -0.63) |
| 1210 | -0.82 | (-1.1, -0.49) |
| 1660 | -0.94 | (-1.3, -0.54) |
| 2390 | -1.6 | (-2.4, -0.85) |

**Comparison of Results of This Study with Other Risk Assessments**

Several studies have made quantitative assessments of the potential risk of lung cancer from diesel exposure based on both bioassay and epidemiological data available. Harris (1983) estimated a range for the carcinogenic potency of DE based on the negative epidemiologic study of London Transport Workers (Waller, 1980). Harris also estimated the potency of DE in humans using a comparative analysis of laboratory and epidemiological data on DE and two chemically related environmental exposures -- coke oven emissions and roofing tar emissions. Smith and Stayner (1990) estimated the potency of DE from a rat bioassay of Mauderly *et al.* (1986). Albert and Chen (1986) made a similar application of the Mauderly *et al.* data; they also utilized a comparative potency approach somewhat similar to that applied by Harris. After reviewing both epidemiological data as well as short-term bioassay data on exposure to several sources of polycyclic aromatic hydrocarbons (PAHs), including DE, Cuddihy *et al.* (1984) projected an upper limit to the carcinogenic potency of DE.

Table 10 summarizes the carcinogenic potencies obtained from these studies, expressed in units of increase in relative risk of lung cancer per $\mu g/m^3$-year exposure to

| Table 10<br>Estimates of the Increase in Relative Risks<br>of Lung Cancer from Exposure to DE | | |
|---|---|---|
| | Method | Increase in Relative Risk ($x10^{+4}$) per $\mu g/m^3$-year[a] |
| Harris (1983) | Epidemiological analysis of London transport workers | MLE[b] = 1.2<br>95% upper bound = 4.3<br>95% lower bound = -1.8 |
| | Average relative potency for extracts from three automobiles | 0.35 |
| | Range from relative potency of extracts from Caterpillar engine | 0.01 - 0.25 |
| Cuddihy et al. (1984) | Review of epidemiological and short-term assay data | $\leq$ 0.46 |
| Albert and Chen (1986) | Multistage model applied to Mauderly et al. (1987) data on rats | MLE[c] = 0.012<br>95% upper bound = 0.039 |
| | Relative potency | 0.050 |
| Smith and Stayner (1990) | Multistage time-to-tumor model applied to Mauderly et al. (1987) data on rats | 0.046 - 0.092 |

[a] Assumes occupational exposure (40 hours/week, 50 weeks/year).
[b] MLE = Maximum Likelihood Estimate.
[c] Albert and Chen (1986) only provided 95% upper bound.

DE. Computations required to express the results from the various studies in these units are provided in Appendix E.

Estimates of the carcinogenic potency of DE obtained from these studies vary by more than two orders of magnitude. The largest estimate is the upper limit of $4.3x10^{-4}$ per $\mu g/m^3$-year obtained by Harris from a negative epidemiological study. The estimates derived from lung cancer induced in long-term animal inhalation studies range from $0.012x10^{-4}$ per $\mu g/m^3$-year to $0.092x10^{-4}$ per $\mu g/m^3$-year. Even though the upper limit derived by Harris is much larger than the estimates derived from the animal data, the

epidemiological data used by Harris are not inconsistent with the animal results because the estimates based on animal data fall between the lower and upper limits estimated by Harris.

To obtain some idea of how these estimates compare with the results of this study, Figure 13 shows a graph of the relative risks measured in the cohort as a function of ARP. Superimposed upon this graph are plots of the relative risks predicted by the upper limit and maximum likelihood estimate (MLE) obtained by Harris from epidemiological data and the largest estimate based upon the animal data. All of these plots are clearly inconsistent with the relative risks from this study plotted as a function of cumulative exposure to ARP. The width of the confidence intervals obtained from this study compared to the excess relative risk predicted by the plots suggests that, due to statistical variability alone, it would be virtually impossible for a study involving the numbers of subjects and range of exposures in the Garshick *et al.* (1988) study to detect an excess risk of the magnitude indicated by the animal studies. However, it appears that it might be possible to demonstrate a statistically significant relationship between DE and lung cancer if the effect of DE is as large as that predicted by the upper limit obtained by Harris from the epidemiological data.

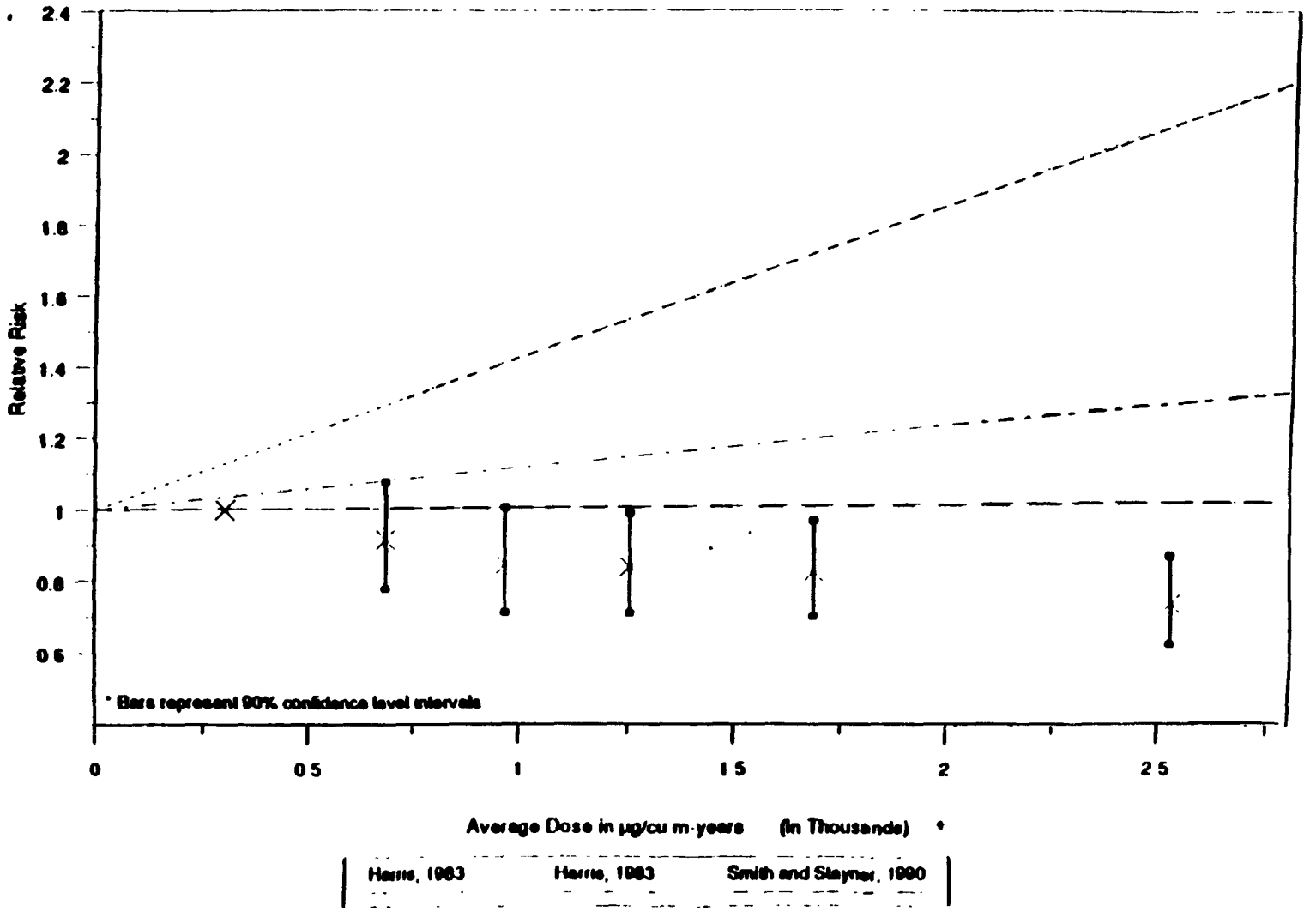
## DISCUSSION

A number of methods of analysis were used to search for a relationship between markers of exposure to DE and lung cancer mortality. Five different markers of diesel exposure were investigated, four of which were adjusted for climatic differences. Measures of exposure studied included cumulative exposure (four or more years in past), as well as cumulative exposure in various time periods in the past (4-8 years, 9-18 years and 18 or more years in the past). If lung cancer mortality from DE is closely associated with exposure in one of these prior time periods, then including exposures in other time periods could mask an effect of diesel exposure.

## Figure 13

**Comparison of Dose Response of Relative Risk Estimated in Other Risk Assessments with Relative Risk Measured in this Study:**

**Climate Adjusted ARP**
**Cumulative Exposure for More Than 3 Years in the Past**



Maximum exposure in any single year more than three years in the past was also analyzed. Consideration of this exposure variable was motivated in part by animal results that indicate that clearance of inhaled particles is less efficient at higher doses, and consequently more intense exposures may be more dangerous per amount inhaled than less intense exposures. No relationship between maximum exposure and lung cancer was found. However, the correlation between this measure of maximum exposure and actual exposures in the population is apt to be poor. Thus, even if more intense exposures were more dangerous per amount inhaled in this cohort, it would probably be difficult to demonstrate this fact since individual measures of DE were not available.

Analyses were conducted using both relative risk and absolute risk models. These methods were applied both to the complete cohort and to subgroups defined by job category in 1959. Single job groups were selected for analysis, as well as combinations of job groups that were thought to have the most reliable exposure information.

None of these analyses showed a pattern that was consistent with an effect of diesel exposure upon lung cancer. Consequently, it was not possible to develop a model for lung cancer mortality as a function of exposure to DE from these data.

There are several potential reasons for this negative finding: 1) the quality of the measures of markers of exposure to DE available may be too poor to reveal an association with lung cancer mortality; 2) confounding with other determinants of lung cancer risk may be masking the effect of DE; 3) there may be no causal relationship between lung cancer risk and exposure to DE, or the relationship may be too small to be detected in a study of this magnitude, given the exposures to diesel experienced by this cohort. Each of these possible causes will be discussed individually below.

**Quality of Exposure Information**

Both the exposure data and the epidemiological data are of critical importance in developing a quantitative dose response model. Deficiencies in either cannot be counterbalanced by exceptional quality in the other.

The exposure data used in this assessment came from air samples collected during a limited time period (between 1981 and 1983) at four small railroads operating in a limited geographical area (northern U.S.). Measured concentrations were of RSP rather than DE *per se*, and these measured concentrations were adjusted to produce markers of

diesel exposure. These data were used to estimate exposures to DE that occurred among railroad workers throughout the U.S. as much as 30 or more years in the past. Clearly, this approach has many potential limitations.

Diesel equipment and working conditions have changed since the 1940s when diesel engines first began being used in large numbers. Woskie *et al.* (1988b) described anecdotal reports of smoky working conditions in the diesel repair shops during the 1950s and 1960s. They also report that limited data available on nitrogen oxide levels during these periods qualitatively support higher levels of DE in the early years of dieselization. By the time samples were collected for this study, these smoky conditions would have been largely mitigated through improved ventilation and the advent of less smoky engines.

Woskie *et al.* report that the types of diesel equipment used by the sampled railroads during the sampling period were representative of the type and ages of locomotives used nationally from the 1950s through the early 1970s. Further, the mixture of diesel equipment used by the four sampled railroads was similar in type and age to what might be expected nationwide. However, there was no way to verify that this mixture was actually representative of conditions nationwide during the period (1945-1980) that the cohort was exposed.

Railroad workers are exposed to emissions from a number of sources that may be confounded with DE in exposure estimates. These include ETS, aerosolized sand, fibrous material, and welding fumes (Hammond *et al.*, 1988). The five markers of diesel exposure used in the analysis have differing degrees of specificity for DE. RSP is the least specific for DE since it does not distinguish between organic and inorganic material. AEM is expected to be the most specific for diesel exposure, since it excludes inorganic material and adjusts for ETS. TEX, on the other hand, includes ETS and could possibly correlate more closely with lung cancer risk than AEM since there is evidence that ETS poses an independent lung cancer risk (EPA, 1990).

There is considerable uncertainty in making geographical distinctions in exposures. The RRB provided an address for a representative of the last railroad for which a cohort member worked, and this address was assumed to correspond to his work location throughout his entire railroad employment. These addresses appeared to correspond

reasonably well to the geographical locations suggested by many of the railroad names, although no systematic study was made. A few of the larger railroads operate in more diverse geographical areas and assigning a single location to these was problematic. For these, the temperature adjustment may work reasonably well for railroads that operate predominately East and West, but more poorly for those that operate predominately North and South.

Given the importance of the exposure estimates to the quantification of risk and the strong effect of temperature upon the exposures measured by Woskie *et al.* (1988), it was considered important to take climatic differences into account in the exposure estimates, insofar as possible. Despite their possible limitations, these estimates should be at least as good, or better, than the estimates that were not climate-adjusted. As a hedge against the possibility that climate-adjusted estimates were biased for some reason, ARP, unadjusted for climate, was also used.

Dieselization of U.S. railroads was virtually complete (95%) by 1959, the first year of follow-up of the cohort (Garshick *et al.*, 1988). However, a significant number of diesel locomotives began to appear during the 1940s, with 14% of U.S. locomotives being diesel by 1947 and 44% by 1951 (AAR, 1989). Moreover, practically the entire cohort first worked for a railroad prior to 1950, and the majority prior to 1946. Thus, because the cohort was only followed for 22 years, because the latency generally associated with chemical or radiation-induced lung cancer is on the order of 20 years or greater, and because early diesel locomotives were likely to have been more smoky than later models, exposures prior to 1959 could be at least as important as exposures subsequent to 1959 in affecting lung cancer incidence in the cohort. Thus, it is important to quantify exposures prior to 1959 even though there is limited information available from this time period.

Exposure levels for a given year prior to 1959 were estimated by the product of the estimated exposure level in 1959 and the percent of the U.S. railroad fleet that was diesel in 1959. Thus, a person was assumed to have worked in the same job prior to 1959 as in 1959. More importantly, exposures were assumed to be equal among all persons in a given job prior to 1959; in reality, those who worked with diesel equipment probably experienced exposures as high (and probably higher, assuming early diesel

locomotives were smokier and ventilation was poorer) as those occurring subsequent to 1959, while those who did not work with diesel equipment were unexposed. Although the approach used in the analysis may result in average exposures during this period that are approximately correct, by not having information on individual exposures, important dose response information may have been obfuscated.

**Potential for Confounding**

Only sketchy information on smoking habits is available for this cohort. Garshick *et al.* (1988) report that, in a survey of 517 current railroad workers, no difference was found in the prevalence of smoking between workers with and without potential exposure to DE. Also, a case control study of lung cancer, based on data provided by the RRB (Garshick *et al.*, 1987), found little difference between crude estimates of effect of diesel exposure and estimates corrected for the effect of smoking.

ETS may also be confounded with DE as a potential cause of lung cancer. Albert *et al.* (1983) compared the potency of DE from several types of engines to that of cigarette smoke condensate in several short-term *in vitro* assays. DE was more potent than cigarette smoke condensate in most, but not all, of these assays. However, there were significant differences in potencies among DE from different types of engines. Most of the data on diesel reported by Albert *et al.* came from experiments with small diesel engines. Data from diesel railroad locomotives were not included in their analysis.

Table 3 suggests that concentrations of ETS smoke were comparable to concentrations of DE in many jobs (the concentration of ETS is estimated as the difference between RSP and ARP). If ETS and exhaust from diesel locomotives have comparable potencies, then it would be difficult to separate their effect on lung cancer. However, in that case, TEX, which is a marker for the combination of DE and ETS, should provide a more consistent correlation with lung cancer than the other markers considered.

**Evidence of an Effect of DE Upon Lung Cancer From This Study**

Numerous analyses discussed elsewhere in this report on the relation between various surrogate measures of diesel exposure and lung cancer mortality failed to establish a relationship between exposure to DE and lung cancer. However, as discussed above, the quantitative measures of diesel exposure used in this study have many

potential shortcomings. Consequently, the negative finding may be due to weaknesses in the exposure data rather than to an absence of diesel effect *per se*.

The principal evidence for an effect of DE upon lung cancer risk comes from analyses in which diesel exposure was dichotomized (yes/no). One type of analysis involved calculating the relative risk in exposed versus unexposed workers for six five-year age intervals based on age in 1959. That analysis has now been conducted in four different ways, two by Garshick *et al.* (1988) (their Tables 5 and 6) and two in the current report (our Tables 5 and 6). These analyses give similar results. They show that the relative risk increases progressively from the null value among workers aged 60-64 in 1959 to a statistically significant value of 1.4 among workers aged 40-44 in 1959.

In these analyses, workers assumed to be unexposed are those who worked as clerks and signalmen in 1959. Both of these groups generally worked away from the exhaust of operating trains. This designation of an unexposed group is generally supported by the exposure estimates of Hammond *et al.* (1988) (see also Table 3, which was derived from the Hammond *et al.* data). Based on AEM, which is the marker that is most specific for diesel exposure, clerks have the lowest estimated exposure (7.2 $\mu g/m^3$). Although the exposure assigned to signalmen (23 $\mu g/m^3$) is higher than three of the remaining ten groups, the estimated exposure of signalmen would appear to be lower than the average estimated exposure over the three broad groupings (engineers and firers; conductors, brakemen and hostlers; shop workers) used in the bulk of the analyses reported herein.

Comparisons of age- and year-specific death rates for all deaths in this cohort with corresponding rates for the general U.S. population strongly suggest that follow-up in this cohort was incomplete beginning in about 1977 and became progressively more so with each succeeding year. However, the higher rates among exposed workers do not appear to be due to any lack of follow-up between 1977 and 1980 because restricting follow-up through the end of 1976 does not change the pattern of relative risks appreciably (Table 6).

On the one hand, this finding is reassuring in that it indicates that perhaps lack of follow-up did not appreciably bias the findings in this study. On the other hand, the fact that the increased risk persists even if follow-up ends in 1976 (the relative risk is higher

in the group in the ages 40-45 in 1959 with follow-up ending in 1976 [1.64] than with follow-up ending in 1980 [1.49]) seems at odds with the 20-year latency generally typical of environmentally induced lung cancer.

When these analyses are performed separately for each category of exposed workers (Table 7), it becomes evident that the observed pattern for the cohort is due primarily to engineers and firers. The relative risks for this group follow the same pattern as observed for the complete cohort, although the dependence on age in 1959 is more pronounced for engineers and firers than for the complete cohort. By contrast, although the relative risks among conductors, brakemen and hostlers are generally elevated, they do not vary monotonically with age in 1959; rather, relative risks are higher in the oldest and youngest groups and lowest among the intermediate age groups. The relative risks of shop workers are not significantly elevated, either overall or in any age group, and there is no obvious pattern of dependence of relative risk on age in 1959.

The pattern of relative risk observed in the different job groups has some features that are not consistent with an effect of diesel exposure upon lung cancer rates in this cohort. Exposures were estimated by Hammond *et al.* (1988) as being higher among shop workers than among any other job group (see Table 3). This is consistent with the fact that shop workers perform their duties near running locomotives in relatively confined areas. Moreover, it is likely that early diesel shops were designed for steam engines and did not ventilate DE efficiently. Woskie *et al.* (1988b) describe anecdotal information and limited historical data on nitrogen oxide and dioxide levels in diesel shops that support higher levels in the shops in the 1950s and 1960s. Since the exposure estimates obtained by Woskie *et al.* and Hammond *et al.* were not adjusted for improvements in ventilation in diesel shops, the excess exposure experienced by the shop workers over other groups was probably greater than estimated from the survey data.

There are several other features of the exposures of shop workers that need to be considered. Some workers classified as shop workers by the ICC job codes worked in non-diesel shops. However, Garshick *et al.* (1988) appear to have selected only job codes involving substantial work around running locomotives. In this regard, they state, "...shop workers had jobs mainly located inside the roundhouse and diesel repair facilities..."

Shop workers were also the group most likely to be exposed to asbestos. Although Garshick *et al.* (1988) did not find an association with potential for asbestos exposure, any effect that was present would tend to increase the risk of shop workers, which would make the lower risk in shop workers even more inconsistent with an effect of diesel exposure in this group.

Thus, shop workers were both likely to have experienced the highest exposures to DE and were the most likely to have been exposed to asbestos. The fact that no excess lung cancer was observed among shop workers suggests that the excess observed among groups exposed to lower levels of DE may not have been a result of exposure to DE.

Although an internal control group (clerks and signalmen in this study) should generally be more appropriate than an external group such as U.S. white males, it is possible that this may not be the case in an observational study such as this, particularly since no information was available on smoking. Clerks, in particular, could differ from engineers, firers, shop workers, etc., physically, socially, and in personal habits, including use of tobacco. Thus, we believe that it was useful to complement the analysis based on internal controls by considering external controls as well. It was this analysis that led us to suspect that follow-up was incomplete in this cohort. In interpreting the comparisons with U.S. white males in Table 5, it should be kept in mind that in addition to the apparent lack of follow-up in the cohort, 11% of known deaths were not assigned a specific cause.

The analysis of cumulative years of exposure was performed to compare to the analysis of relative risk with years of exposure performed by Garshick *et al.* (1988). Both analyses used the same intervals to characterize cumulative years of exposure. However, whereas we used a Poisson regression model, the analysis by Garshick *et al.* (1988) utilized a Cox regression model in which calendar year was used to define risk sets for the partial likelihood and age was modelled as a continuous variable. The results of these analyses were quite different; whereas Garshick *et al.* (1988) found relative risk to be increasing with increasing years of exposure, we found a decreasing trend.

The reasons for these differences are not clear. Because of software limitations we were not able to reproduce the Garshick *et al.* (1988) Cox regression analysis. However, we were able to study elapsed time from 1959 until retirement or death in a

Cox regression analysis. (Elapsed time coincides with cumulative years of exposure for workers who did not have a break in their period of employment or change jobs.) This analysis gave marginally significant increases in relative risk for each level of elapsed time; however, no trend with elapsed time was apparent. The fact that we got the same results for elapsed time in a Cox regression analysis and a Poisson regression analysis suggests that the differences between our results and those of Garshick *et al.* (1988) for cumulative years of exposure are not due to differences between Cox regression and Poisson regression, *per se*. Although the reason for these differences has not been completely determined, it appears to be related to the fact that the three variables used in the analysis -- calendar year, age, and cumulative exposure -- are likely to be all highly correlated, which could make the results for cumulative exposure highly sensitive to the specific methods used for controlling for age and time.

## CONCLUSIONS

No relationship between measures of diesel exposure and lung cancer mortality is demonstrated in this study. However, there are many limitations in using the data on markers of diesel exposure collected by Woskie *et al.* (1988a) and Hammond *et al.* (1988) to estimate exposures in the cohort of railroad workers studied by Garshick *et al.* (1988). These limitations are potentially of sufficient magnitude to obscure any relationship between exposure to DE and lung cancer that may exist in the cohort.

The higher relative risk of lung cancer among exposed workers relative to unexposed workers reported by Garshick *et al.* (1988) was verified in the present study. Related findings resulting from our work are as follows:

- The risk of lung cancer among engineers and firers was significantly elevated relative to that of unexposed railroad workers, and the variation in relative risk with age in 1959 (increasing risk with decreasing age) is consistent with DE being responsible for the observed excess.

- Although the risk of lung cancer among conductors, brakemen and hostlers was significantly elevated relative to that of unexposed railroad workers, the variation in risk with age in 1959 (higher at older and younger ages than at intermediate ages) is not consistent with an effect of DE.

- The risk of lung cancer among shop workers was not significantly elevated relative to that of unexposed railroad workers. This is not consistent with an effect of exposure to DE upon lung cancer risk since it is likely that shop workers had significantly higher exposures to DE than either of the other two groups of exposed workers.

- Both internal evidence and comparisons with U.S. mortality rates suggest that, beginning in about 1977, a significant number of deaths occurring in this cohort went undetected.

In this study, follow-up of railroad workers mortality extends through 1980, which is only 22 years from when dieselization of the U.S. railroads was essentially complete. Since the time from first exposure until evidence of an increased risk of environmentally induced lung cancer is often on the order of 20 years, the full impact of any effect of DE upon lung cancer may not be captured by the current study. Moreover, there is evidence that a sizable percent of the total deaths occurring in this cohort between 1977 and 1980 may not have been identified. Thus, it would be worthwhile to conduct a new study of this cohort to take advantage of several additional years of follow-up now available. If such a study is conducted, it is recommended that vital status be verified independently of RRB records.

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# REFERENCES


Albert R, Chen C. 1986. U.S. EPA diesel studies on inhalation hazards. In: Carcinogenicity and Mutagenicity of Diesel Engine Exhaust. Ishinishi N, et al., eds. Elsevier, New York. pp. 411-419.

Albert RE, Lewtas J, Nesnow S, Thorslund TW, Anderson E. 1983. Comparative potency method for cancer risk assessment: Application to diesel particulate emissions. Risk Anal 3:101-117.

Association of American Railroads (AAR). 1989. Railroad Facts. AAR, Washington, DC.

BEIR IV. 1988. Health Risks of Radon and Other Internally Deposited Alpha-emitters. Report of the Committee on the Biological Effects of Ionizing Radiation. National Academy of Sciences, Washington, DC.

BEIR V. 1990. Health Effects of Exposure to Low Levels of Ionizing Radiation. Report of the Committee on the Biological Effects of Ionizing Radiation. National Academy of Sciences, Washington, DC.

Cox DR. 1972. Regression models and life tables (with discussion). J R Statist Soc [Ser B] 34:187-220.

Cox DR, Lindley DV. 1974. Theoretical Statistics. Chapman and Hall, London.

Crump K. 1984. An improved procedure for low-dose carcinogenic risk assessment from animal data. J Environ Pathol Toxicol 5:339-348.

Crump K, Allen B. 1985. Methods for quantitative risk assessment using occupational studies. Amer Stat 39(Pt.2):442-450.

Crump K, Howe R. 1984. The multistage model with a time-dependent dose pattern: applications to carcinogenic risk assessment. Risk Anal 4:163-176.

Cuddihy RG, Griffith WC, McClellan RO. 1984. Health risks from light duty diesel vehicles. Environ Sci Technol 18:14A-21A.

Environmental Protection Agency (EPA). 1990. Health effects of passive smoking: Assessment of lung cancer in adults and respiratory disorders in children. EPA/600/6-90/006A. Office of Health and Environmental Assessment, Office of Atmospheric and Indoor Air Programs, Washington, DC.

Ferguson BV. 1990. Personal communication. Director, Bureau of Research and Employment Accounts, Railroad Retirement Board, Chicago, IL.

Garshick E. 1991. Personal communication. Brockton Veterans Administration Medical Center, Brockton, MA.

Garshick E, Schenker M, Junoz A, et al. 1987. A case-control study of lung cancer and diesel exhaust exposure in railroad workers. Am Rev Respir Dis 135:1242-1248.

Garshick E, Schenker M, Munoz A, et al. 1988. A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. Am Rev Respir Dis 137:820-825.

Hall NEL, Wynder EL. 1984. Diesel exhaust exposure and lung cancer: A case-control study. Environ Res 34:77-86.

Hammond S, Smith T, Woskie S, Leaderer B, Bettinger N. 1988. Markers of exposure to diesel exhaust and cigarette smoke in railroad workers. Am Ind Hyg Assoc J 49:516-522.

Harris J. 1983 Diesel emissions and lung cancer. Risk Anal 3:83-100.

Howe G, Fraser D, Lindsay J, Presnal B, Yu S. 1983. Cancer mortality (1965-1977) in relation to diesel fume and coal exposure in a cohort of retired railway workers. J Natl Cancer Inst 70:1015-1020.

Interstate Commerce Commission (ICC). 1951. Bureau of Transport Economics and Statistics. List of occupations or positions in railroad service, file 21-B-2. USGPO, Washington, DC.

Kaplan I. 1959. Relationships of noxious gases to carcinoma of the lung in railroad workers. J Am Med Assoc 171:2039-2043.

Lewtas J, Williams K. 1986. A retrospective view of the value of short-term genetic bioassays in predicting the chronic effects of diesel soot. In: Carcinogenicity and Mutagenicity of Diesel Engine Exhaust. Ishinishi N, et al., eds. Elsevier, New York. pp. 119-140.

Mauderly JL, Jones RK, McClellan RO, et al. 1986. Carcinogenicity of diesel exhaust inhaled chronically by rats. In: Carcinogenicity and Mutagenicity of Diesel Engine Exhaust. Ishinishi N, et al., eds. Elsevier, New York. pp. 397-410.

McClellan RO. 1987. Health effects of exposure to diesel exhaust particles. Ann Rev Pharmacol Toxicol 27:279-300.

Morrow PE. 1988. Possible mechanisms to explain dust overloading of the lungs. Fund Appl Toxicol 10:369-384.

National Oceanic and Atmospheric Administration (NOAA). 1978. Climate of the States. No. 60. NOAA, Asheville, NC.

Oliver JE. 1987. The Encyclopedia of Climatology. Van Nostrand Reinhold Company, New York. p. 833.

Preston DL, Lubin JH, Pierce DA. 1990. EPICURE. Programs for Regression Analyses of Epidemiologic Data Including: AMFIT, PECAN, G'MBO, PEANUTS and DATAB.

Smith R, Stayner L. 1990. An exploratory assessment of the risk of lung cancer associated with exposure to diesel exhaust based on a study in rats. Division of Standards Development and Technology Transfer, National Institute for Occupational Safety and Health.

Waller R. 1980. Trends in lung cancer in London in relation to exposure to diesel fumes. In: Health Effects of Diesel Engine Emissions. Vols 1 and 2. Pepelko W, et al., eds. U.S. Environmental Protection Agency, Cincinnati, OH. pp. 1085-1097.

Waxweiler R, Wagoner J, Archer V. 1973. Mortality of potash workers. J Occup Med 15:486-489.

Wong O, Morgan R, Keifets L, et al. 1985. Mortality among members of a construction equipment operators union with potential exposure to diesel exhaust emissions. Br J Ind Med 42:435-448.

Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. 1988a. Estimation of the diesel exhaust exposures of railroad workers: I. Current exposures. Am J Ind Med 13:381-394.

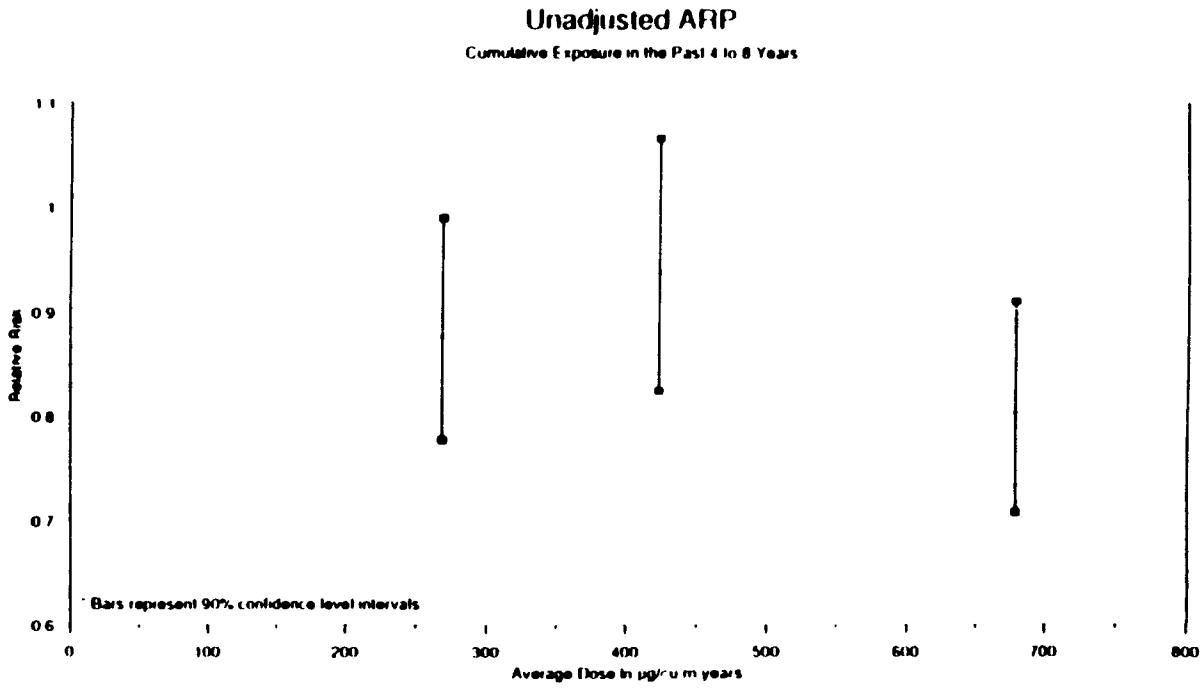
Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. 1988b. Estimation of the diesel exhaust exposures of railroad workers: II. National and historical exposures. Am J Ind Med 13:395-404.

# APPENDIX

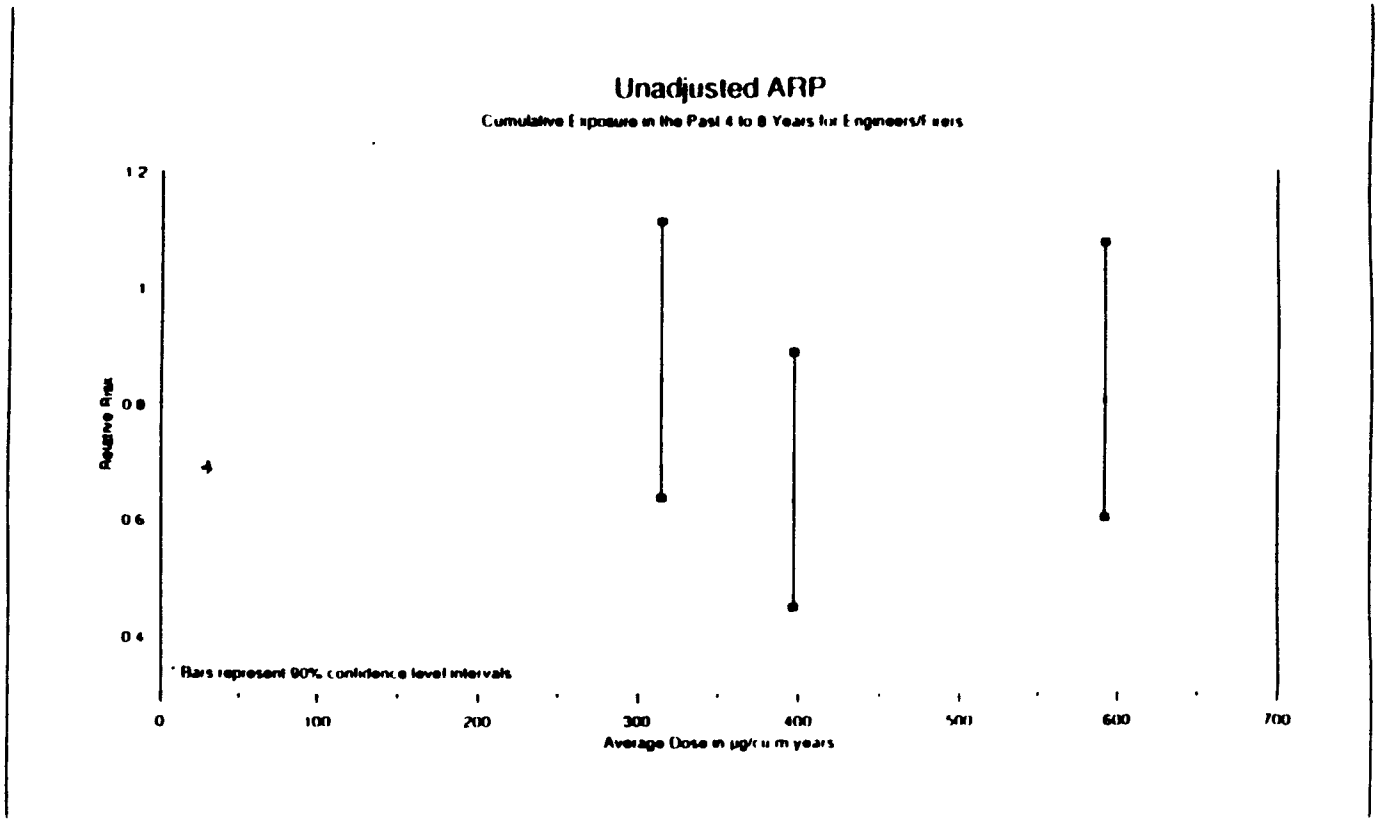
# GRAPHS OF RELATIVE RISK FOR VARIOUS EXPOSURE MEASURES AND SUBSETS OF THE COHORT

This the appendix contains figures for the risk assessment presented in Appendix B. In these figures, relative risk is plotted against the average dose for several dose categories. Figures are provided for each of the five markers for DE and for exposure to DE accumulated over four time periods: more than three years in the past, from four to eight years in the past, from nine to 18 years in the past, and more than 18 years in the past. Figures are provided for the complete cohort and for engineers/firers, conductors/brakemen, and shop workers separately.

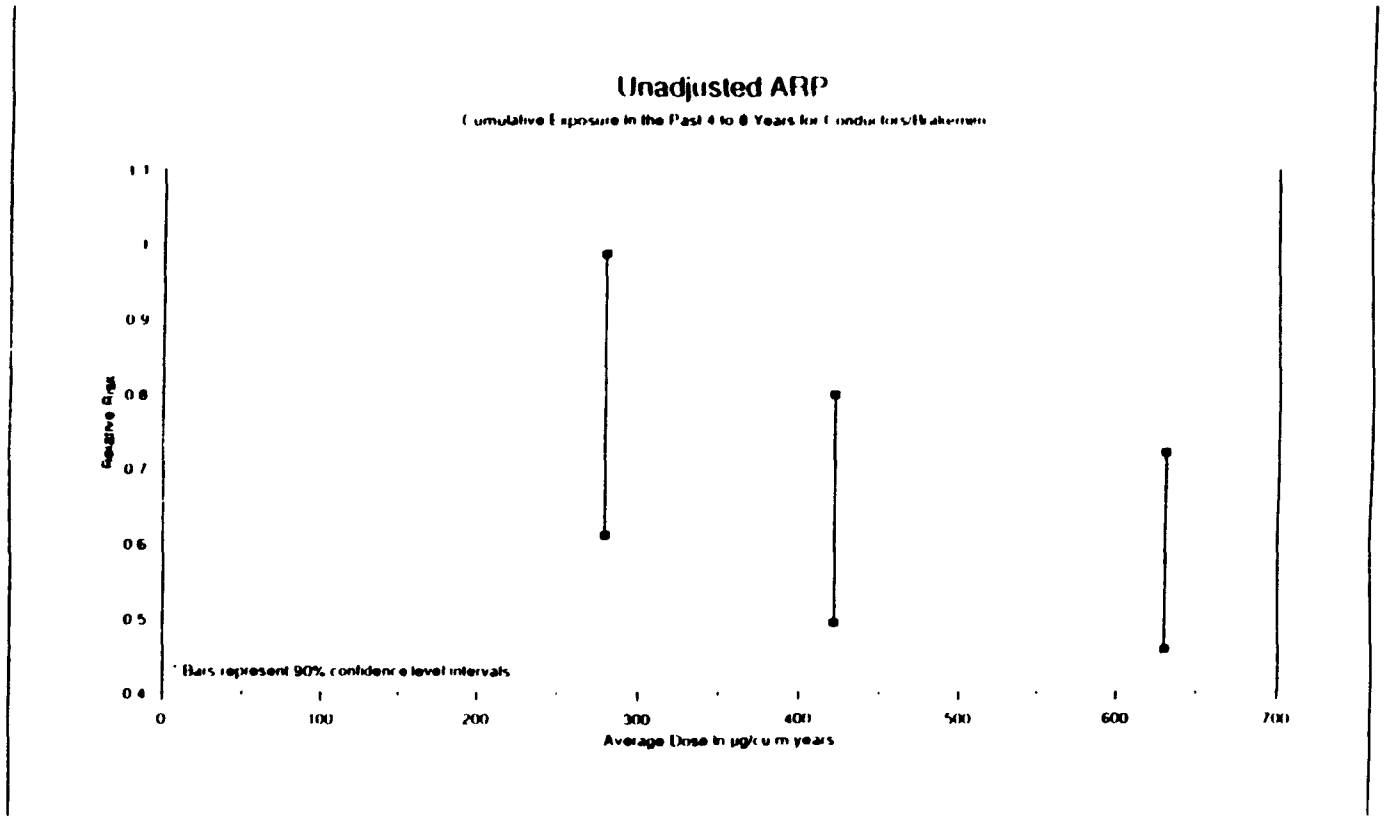
# Figure A1.1


Unadjusted ARP
Cumulative Exposure in the Past 4 to 8 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

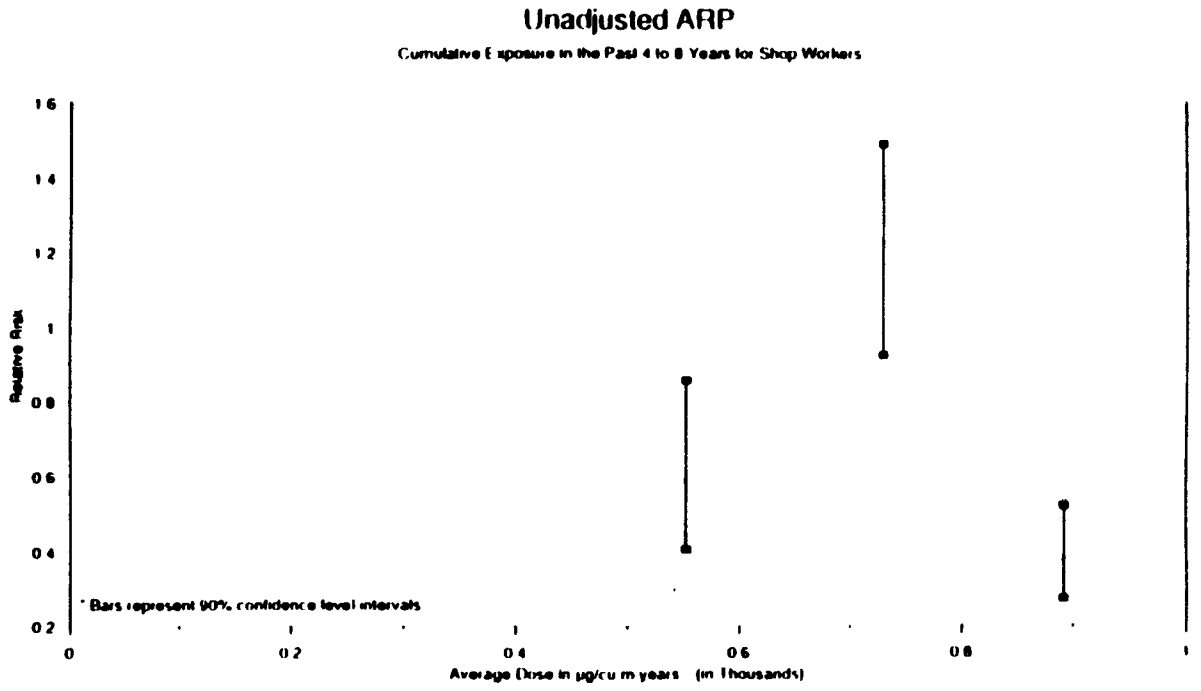
# Figure A1.2


Unadjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Engineers/Fitters
Relative Risk
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
Average Dose in µg/cu m years

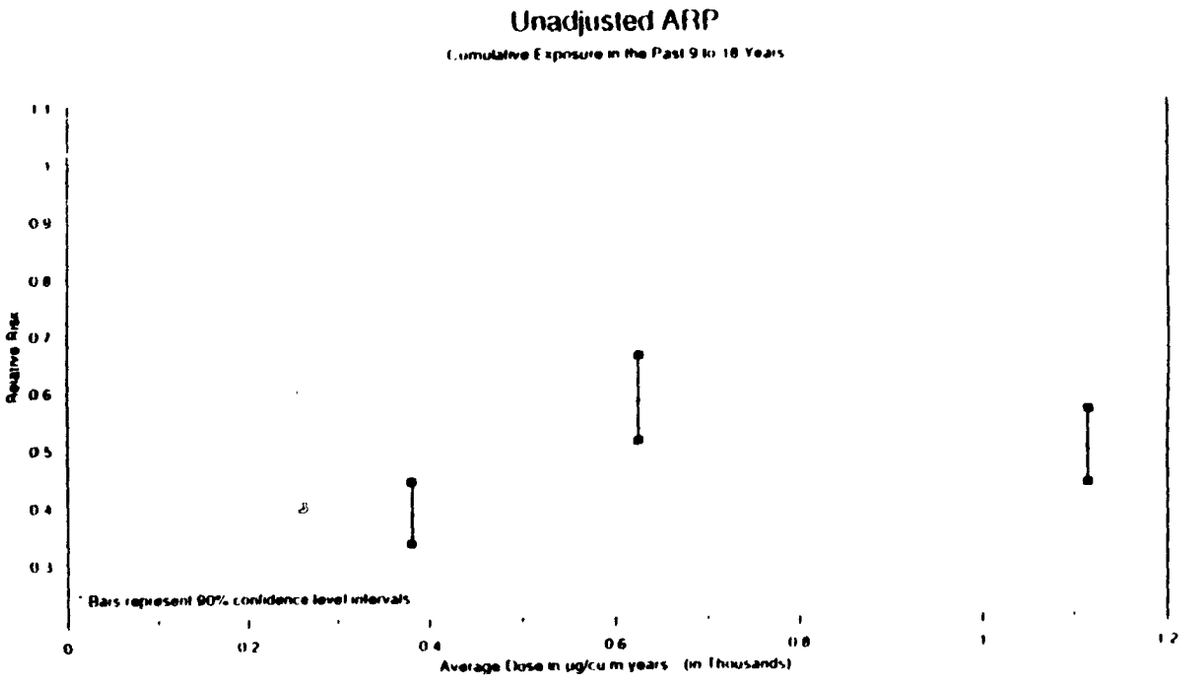
# Figure A1.3


Unadjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
Average Dose in µg/cu m years

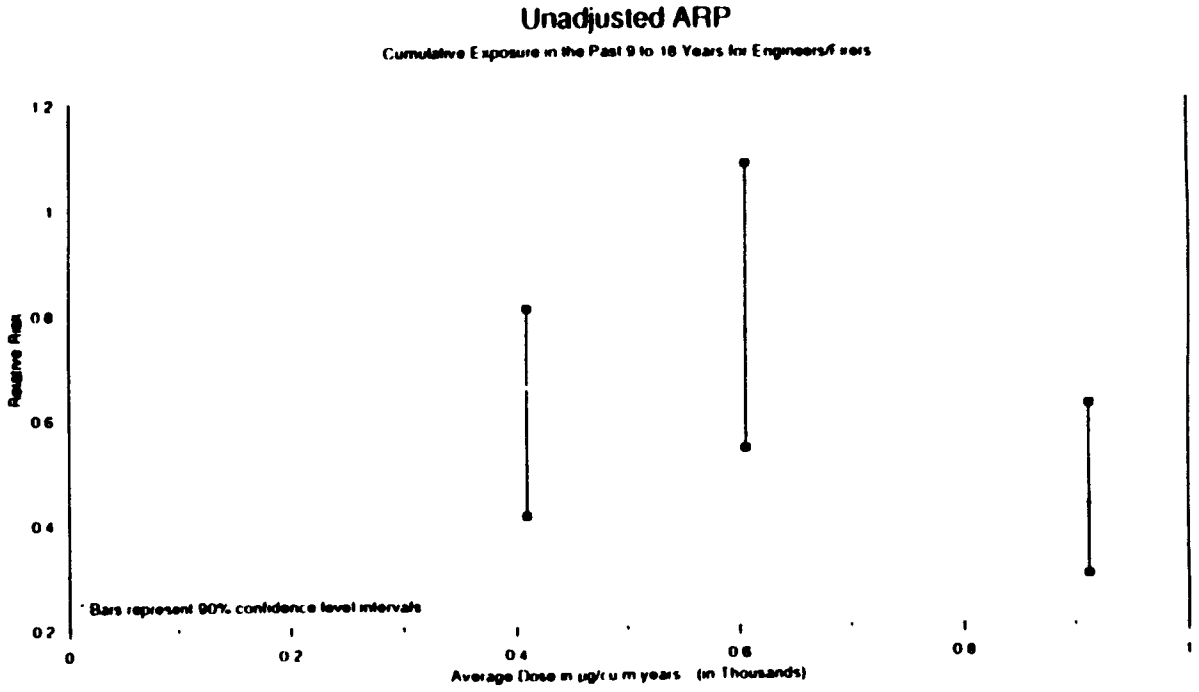
# Figure A1.4


Unadjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Shop Workers
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

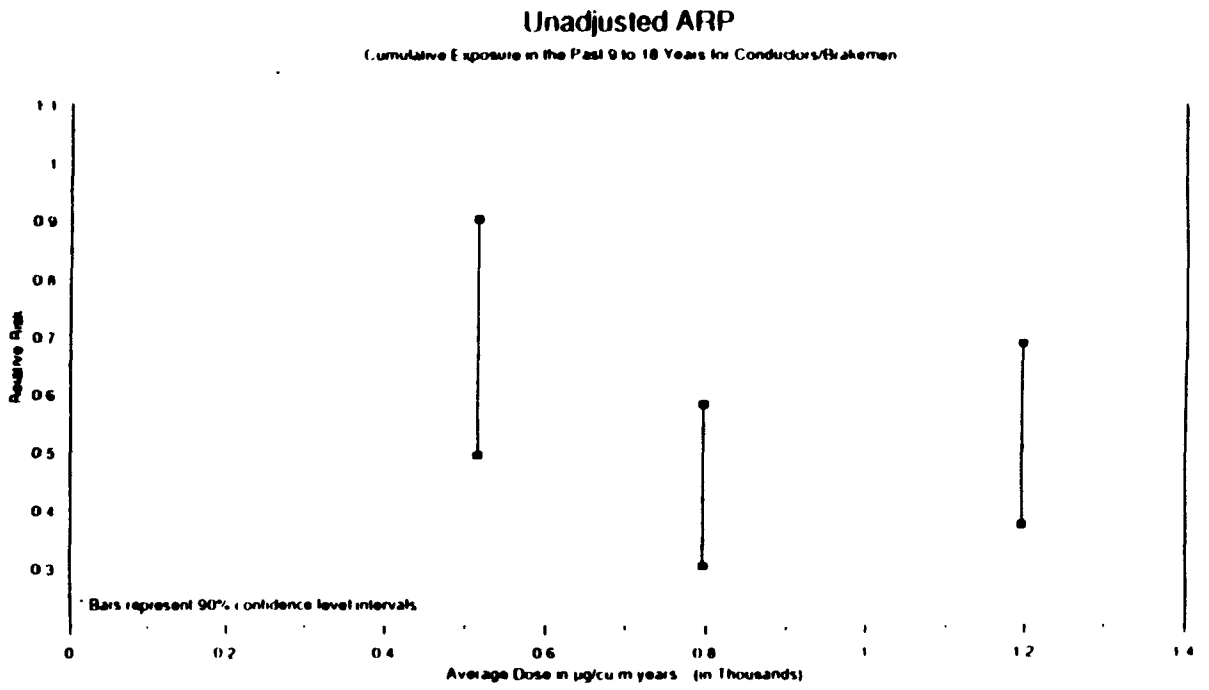
# Figure A1.5


Unadjusted ARP
Cumulative Exposure in the Past 9 to 18 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

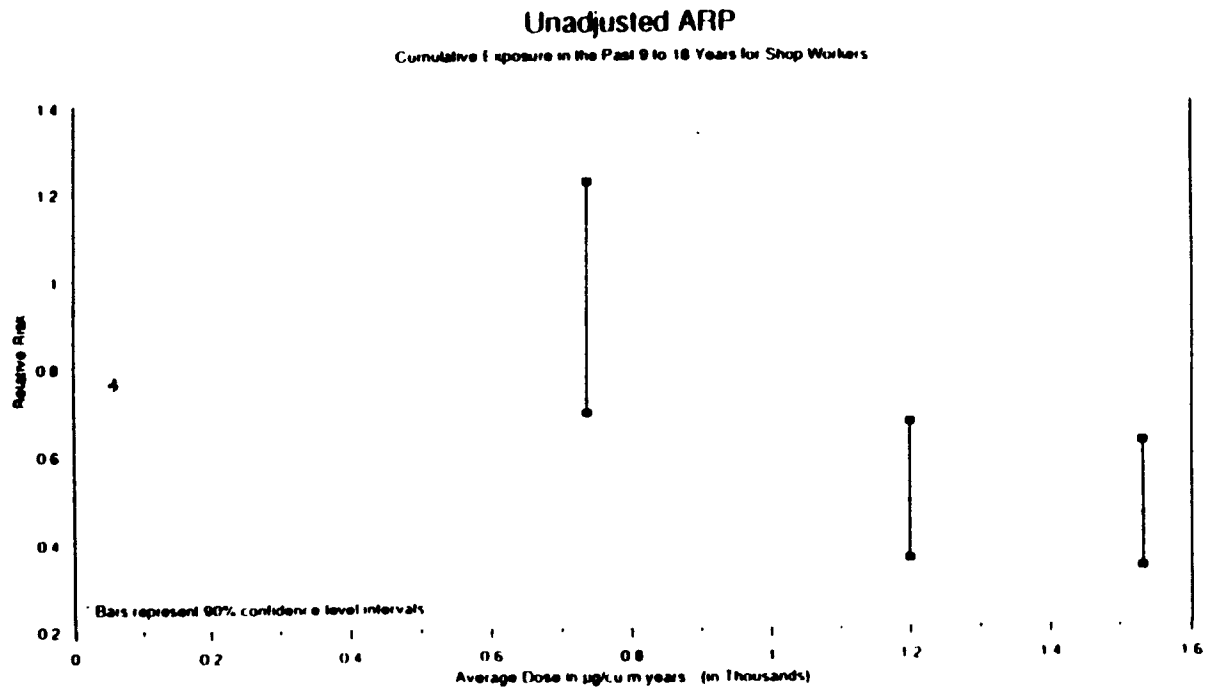
# Figure A1.6


Unadjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Engineers/Fitters
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

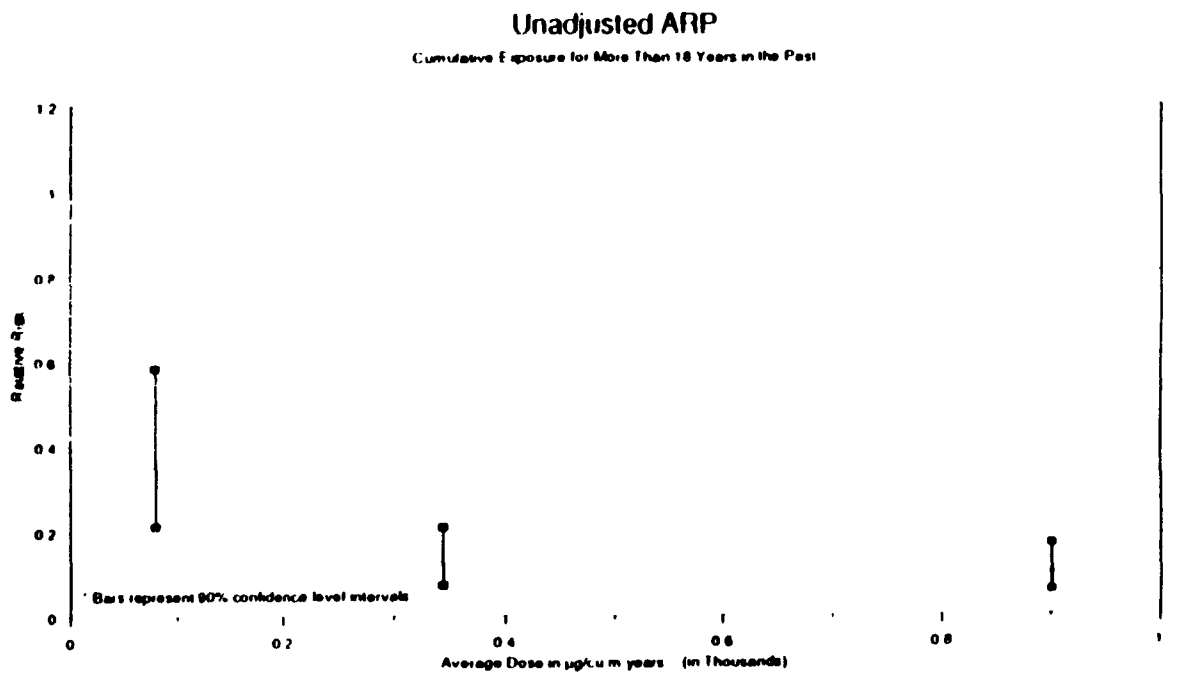
# Figure A1.7


Unadjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average Dose in µg/cu m years (in Thousands)

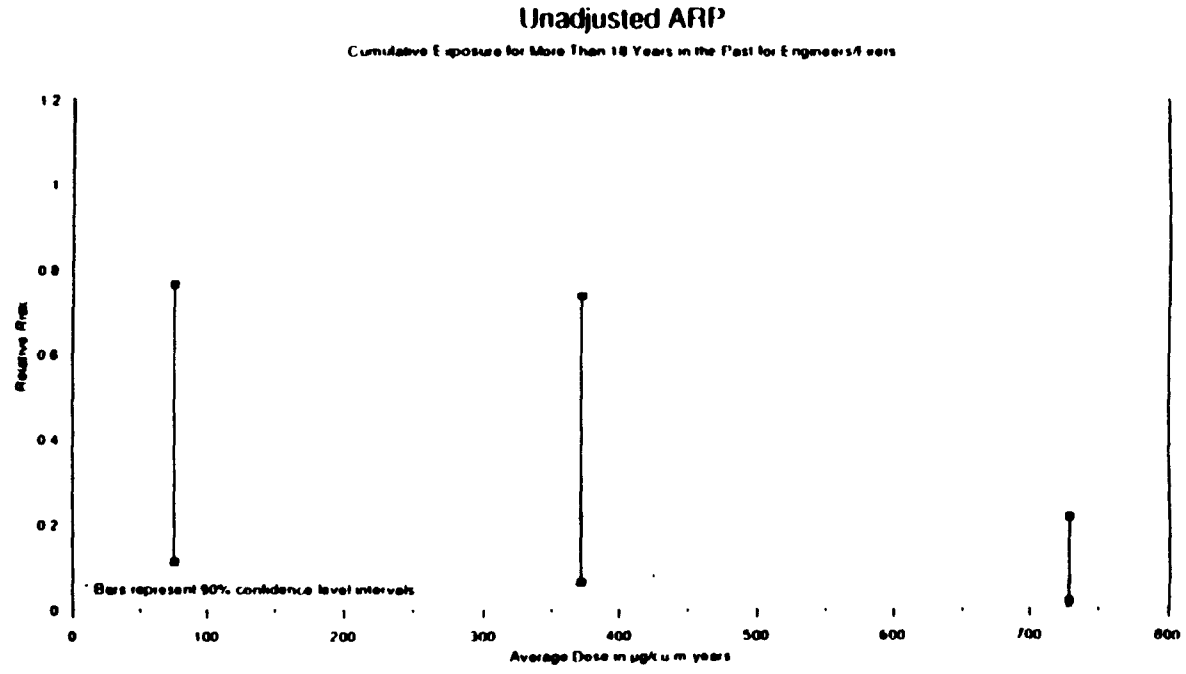
# Figure A1.8


Unadjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Shop Workers
Relative Risk
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Average Dose in µg/cu m years (in Thousands)
Bars represent 90% confidence level intervals

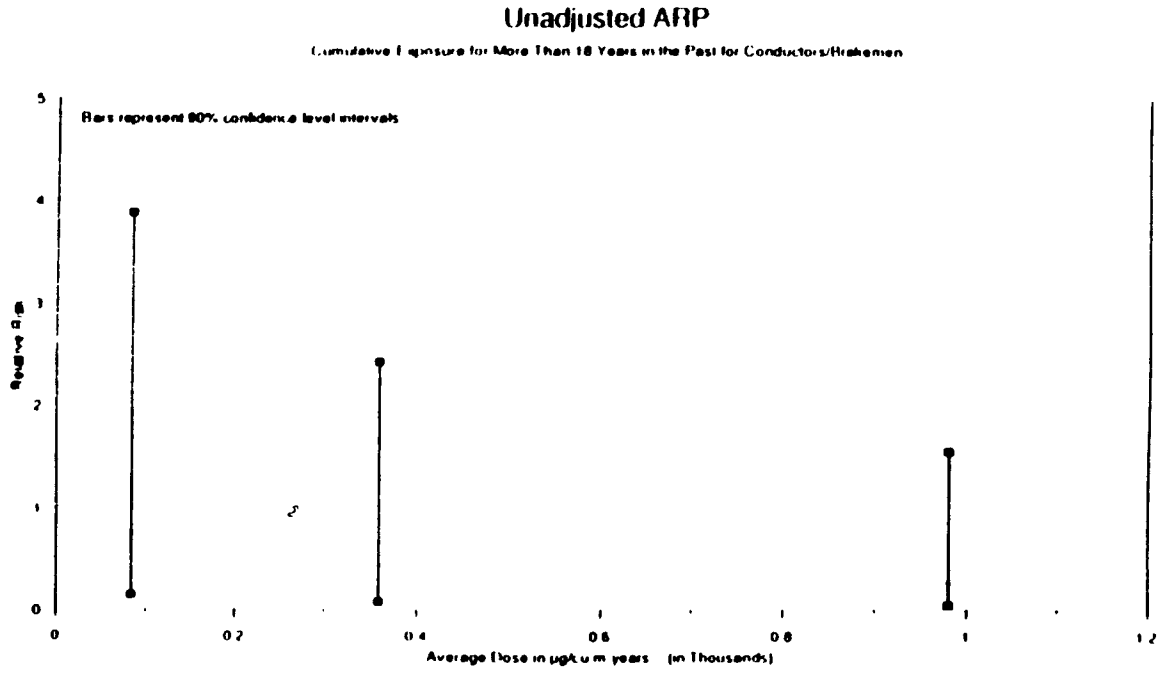
# Figure A1.9


Unadjusted ARP
Cumulative Exposure for More Than 18 Years in the Past
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

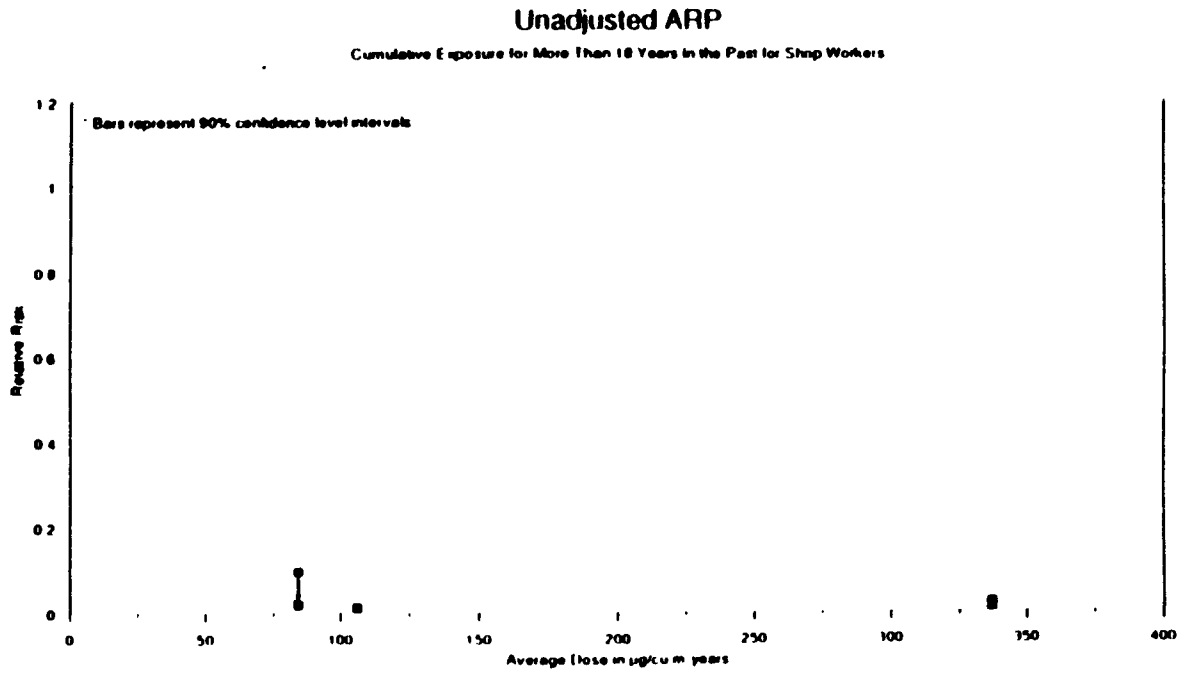
# Figure A1.10


Unadjusted ARP
Cumulative Exposure for More Than 18 Years in the Past for Engineers/Fixers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

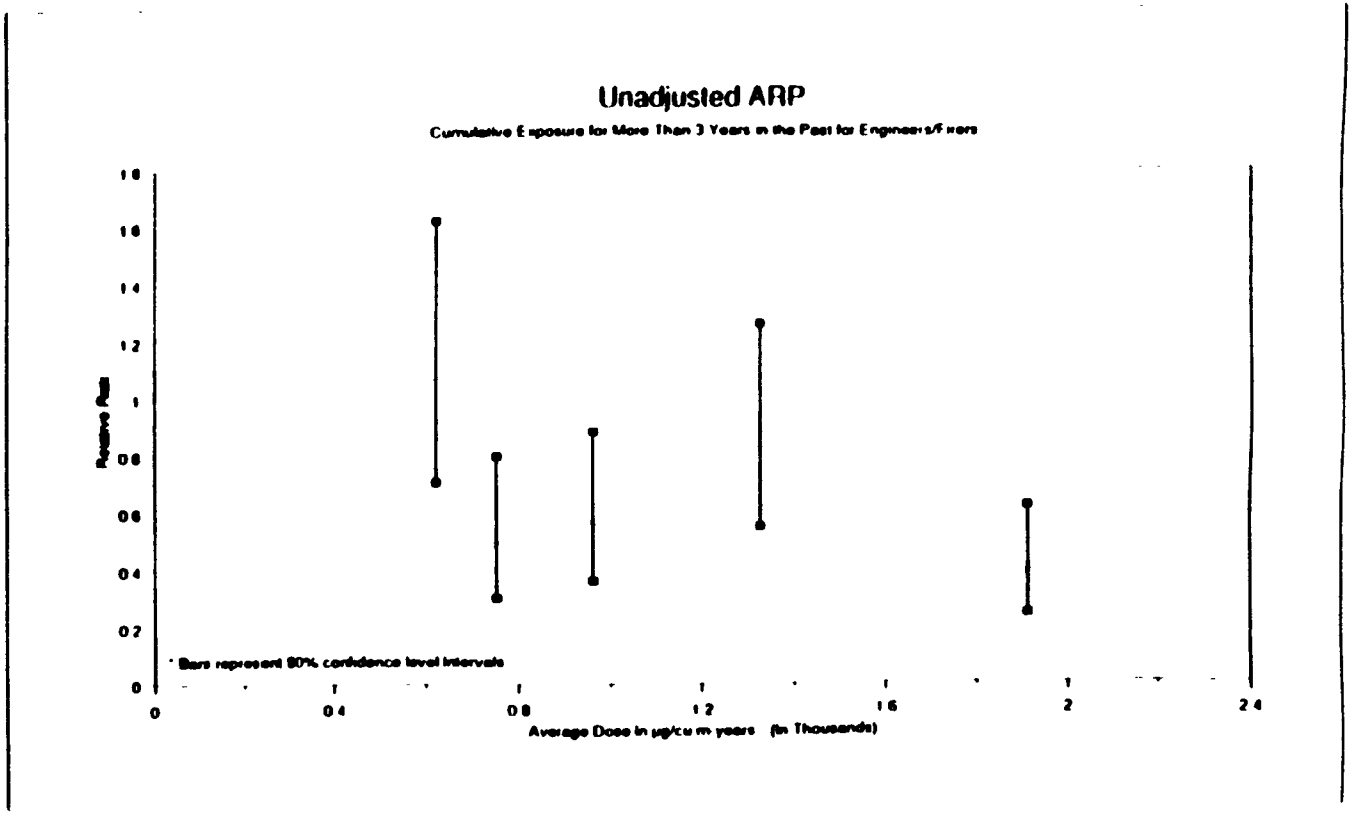
# Figure A1.11


Unadjusted ARP
Cumulative Exposure for More Than 18 Years in the Past for Conductors/Brakemen
Bars represent 90% confidence level intervals
Relative Risk
0
1
2
3
4
5
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

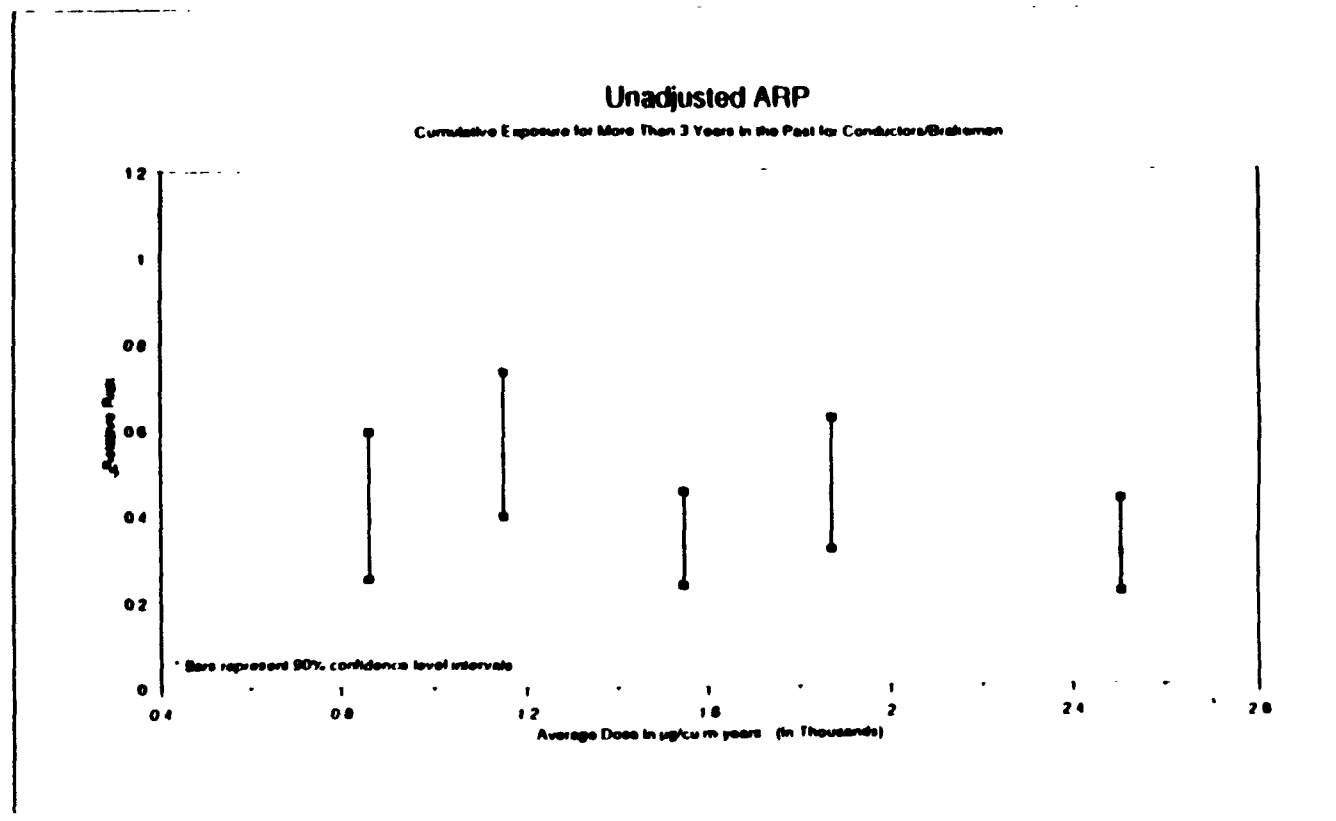
# Figure A1.12


Unadjusted ARP
Cumulative Exposure for More Than 18 Years in the Past for Ship Workers
Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
350
400
Average Dose in µg/cu m years

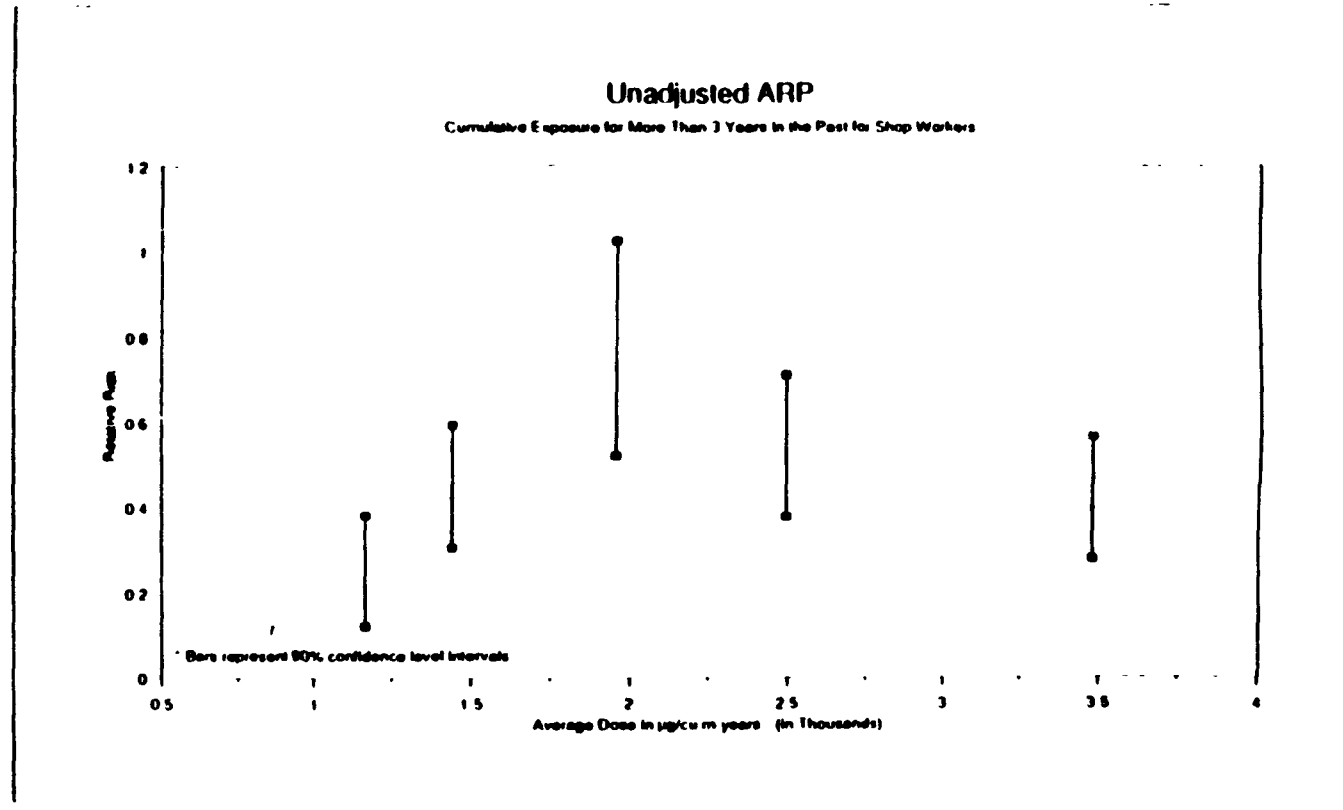
# Figure A1.13


Unadjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Engineers/Firers
Bars represent 90% confidence level intervals
Average Dose in µg/cu m years (in Thousands)

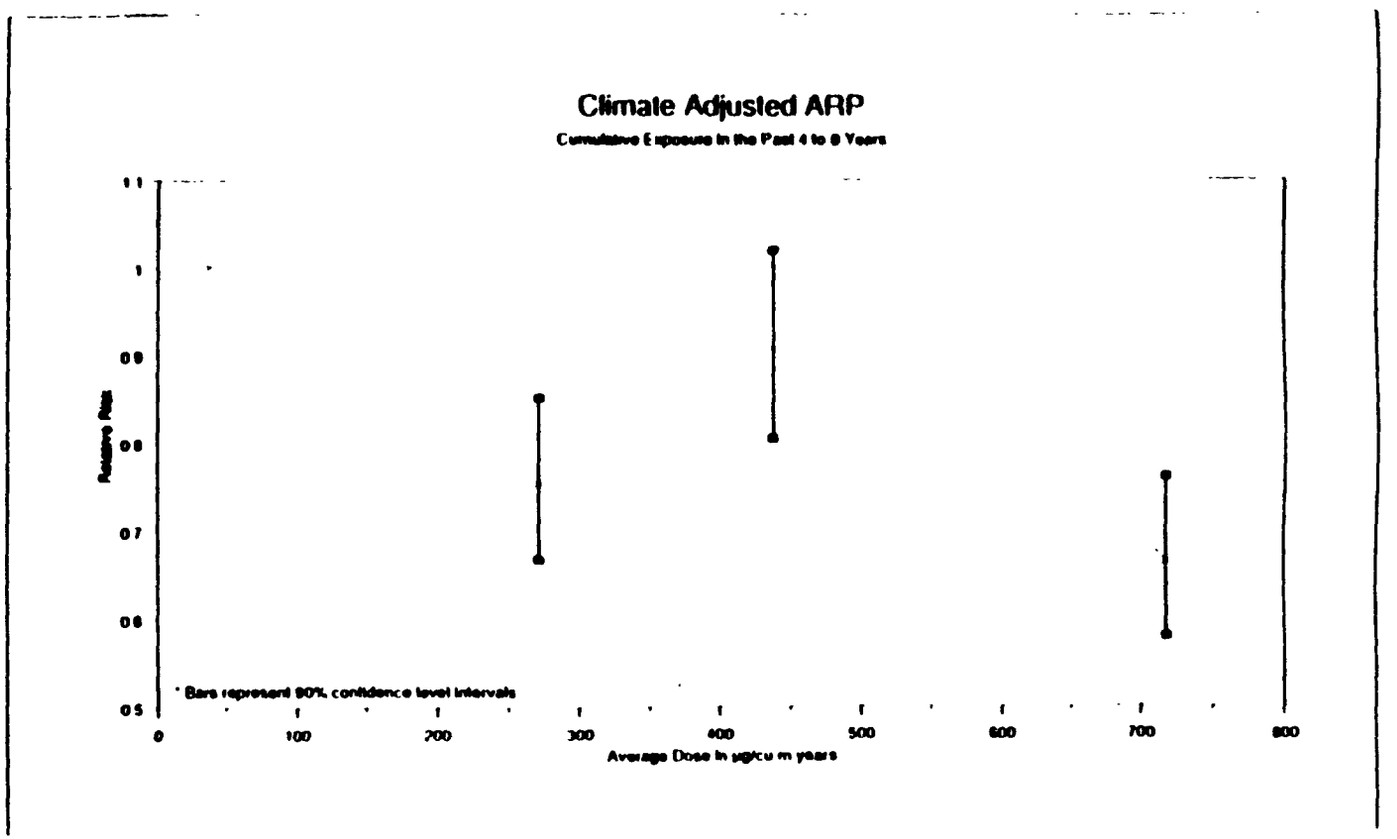
# Figure A1.14


Unadjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Conductors/Brakemen
1.2
1
0.8
0.6
0.4
0.2
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Average Dose in µg/cu m years (in Thousands)
* Bars represent 90% confidence level intervals

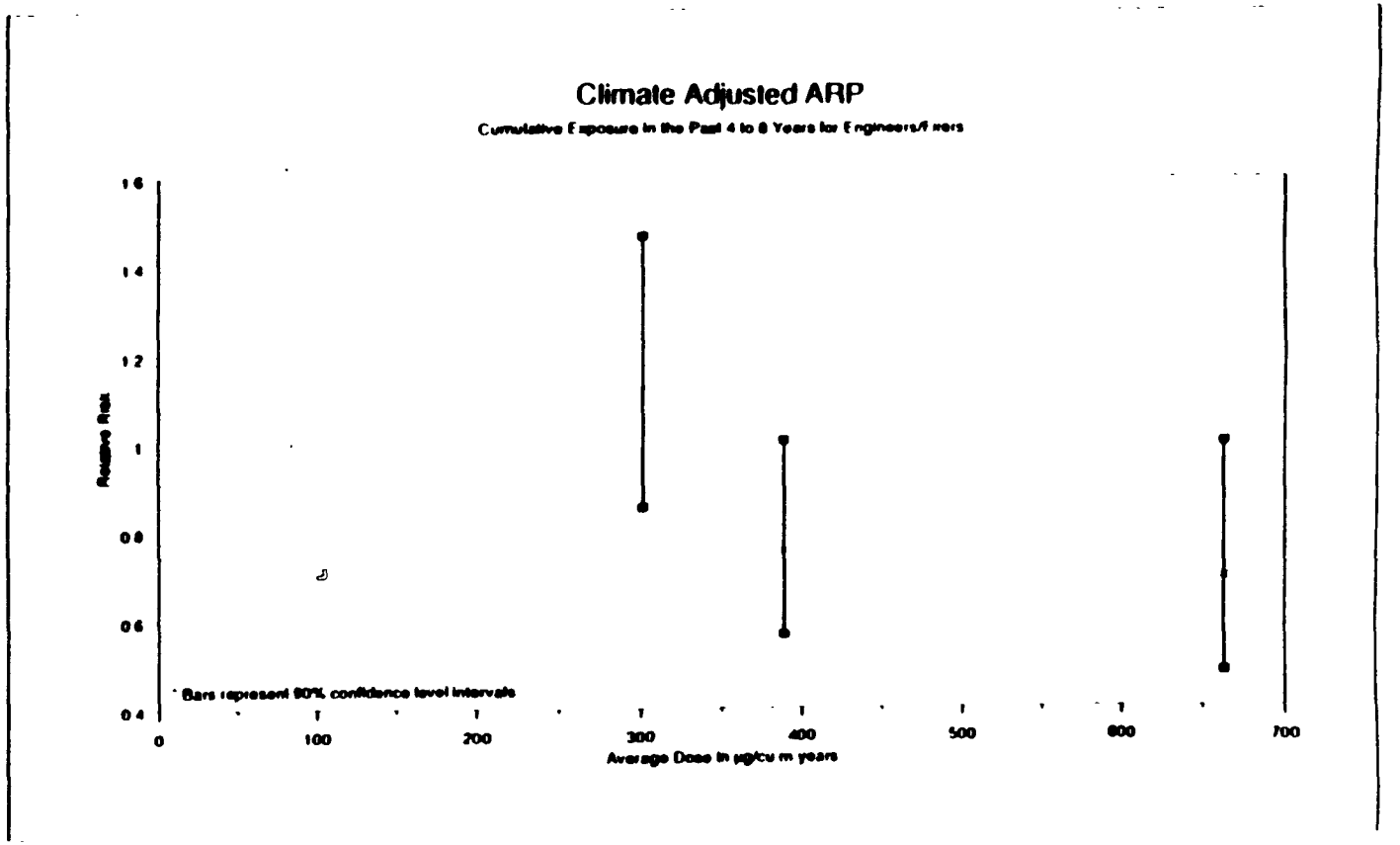
# Figure A1.15


Unadjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Shop Workers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0.5
1
1.5
2
2.5
3
3.5
4
Average Dose in µg/cu m years (in Thousands)

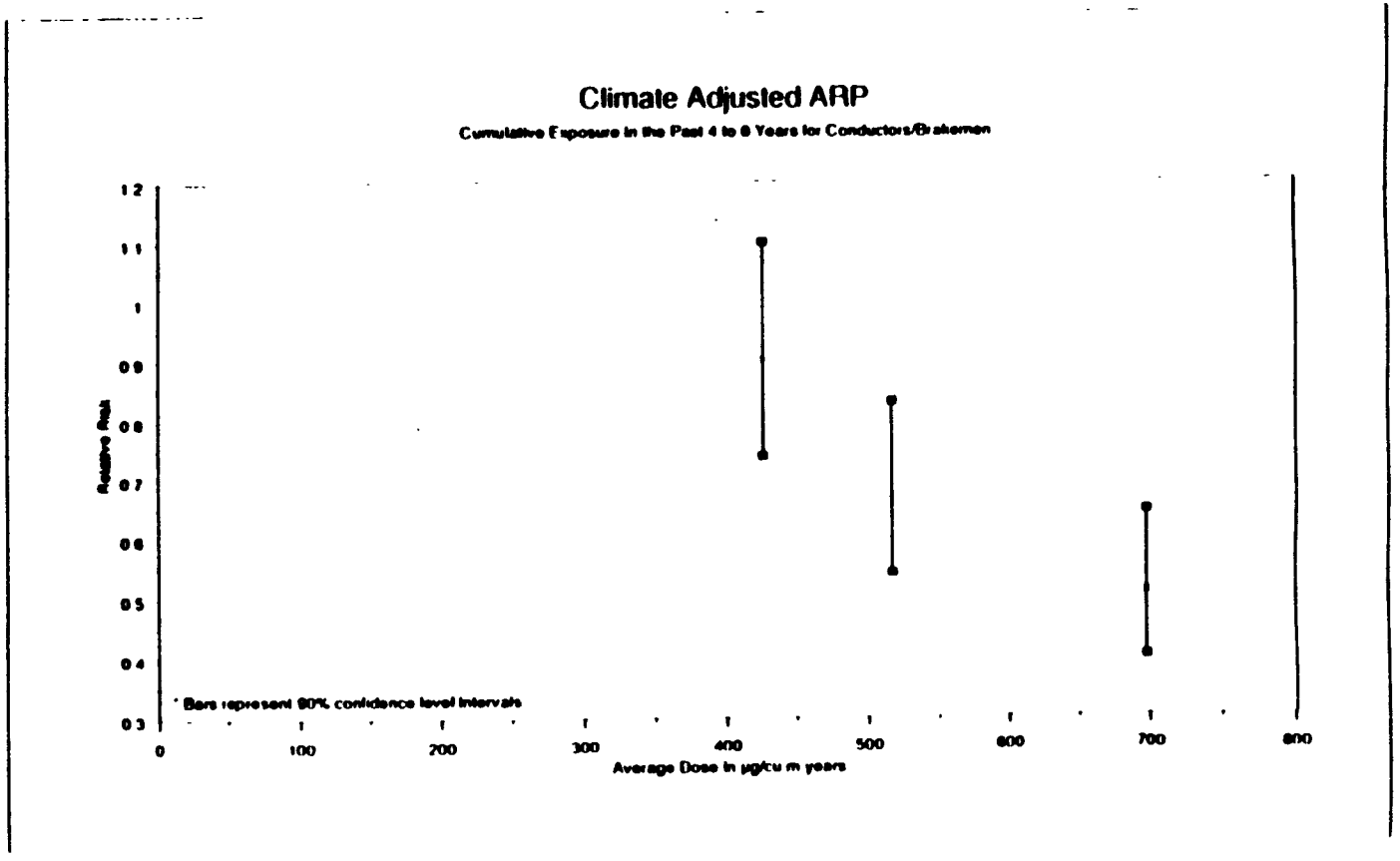
# Figure A2.1


Climate Adjusted ARP
Cumulative Exposure in the Past 4 to 8 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

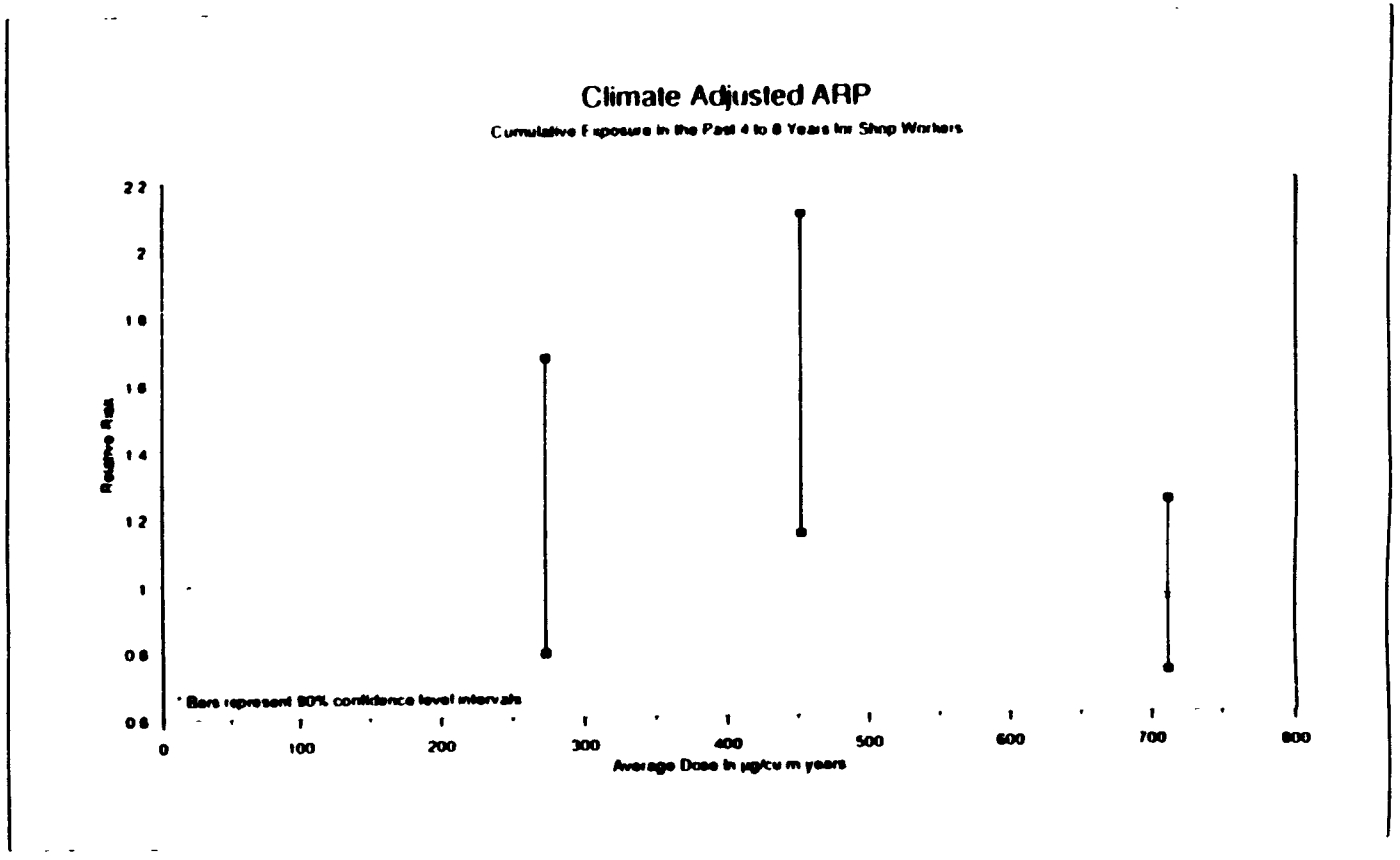
# Figure A2.2


Climate Adjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Engineers/Foremen
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
Average Dose in µg/cu m years

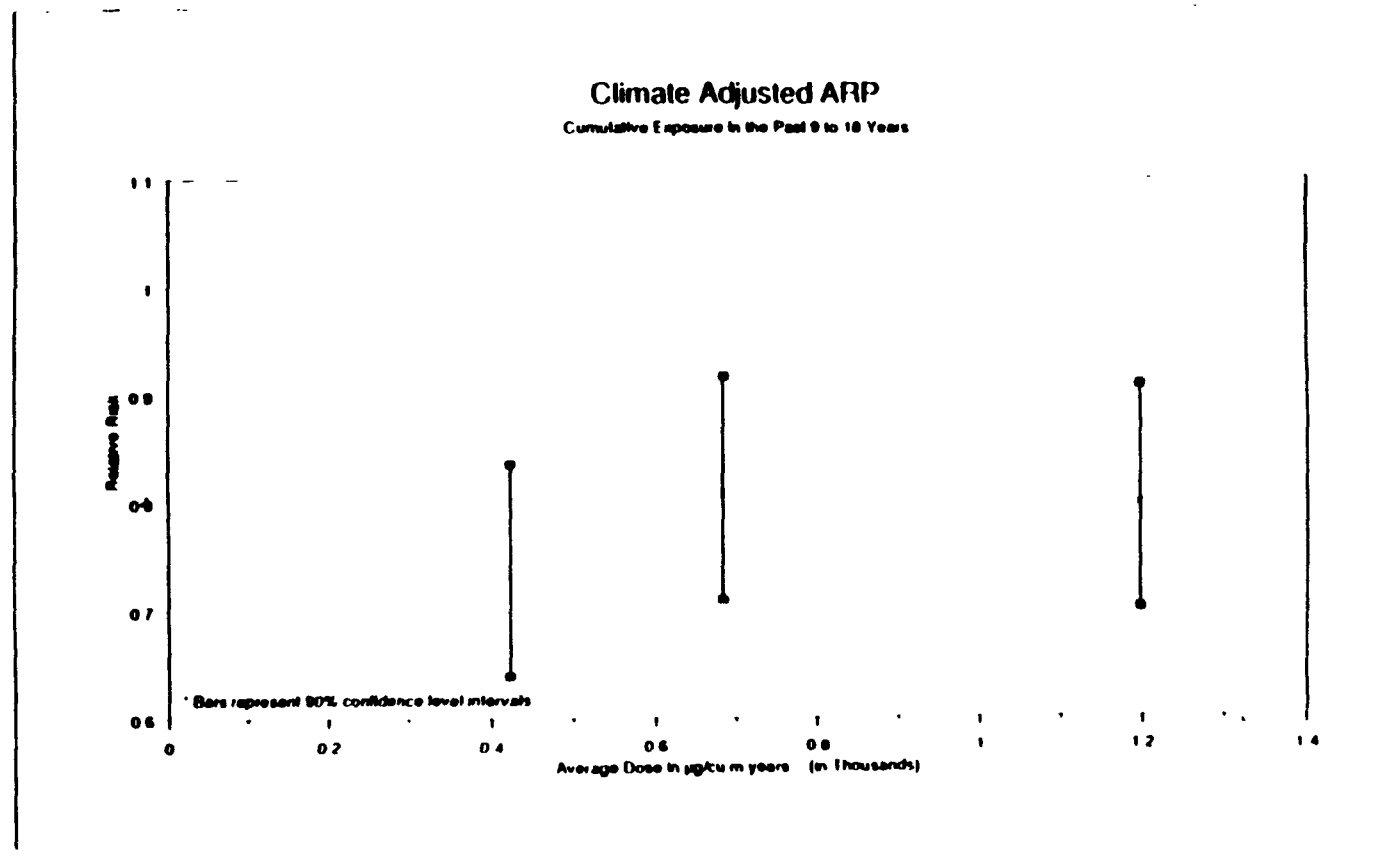
# Figure A2.3


Climate Adjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Conductors/Brakemen
Relative Risk
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

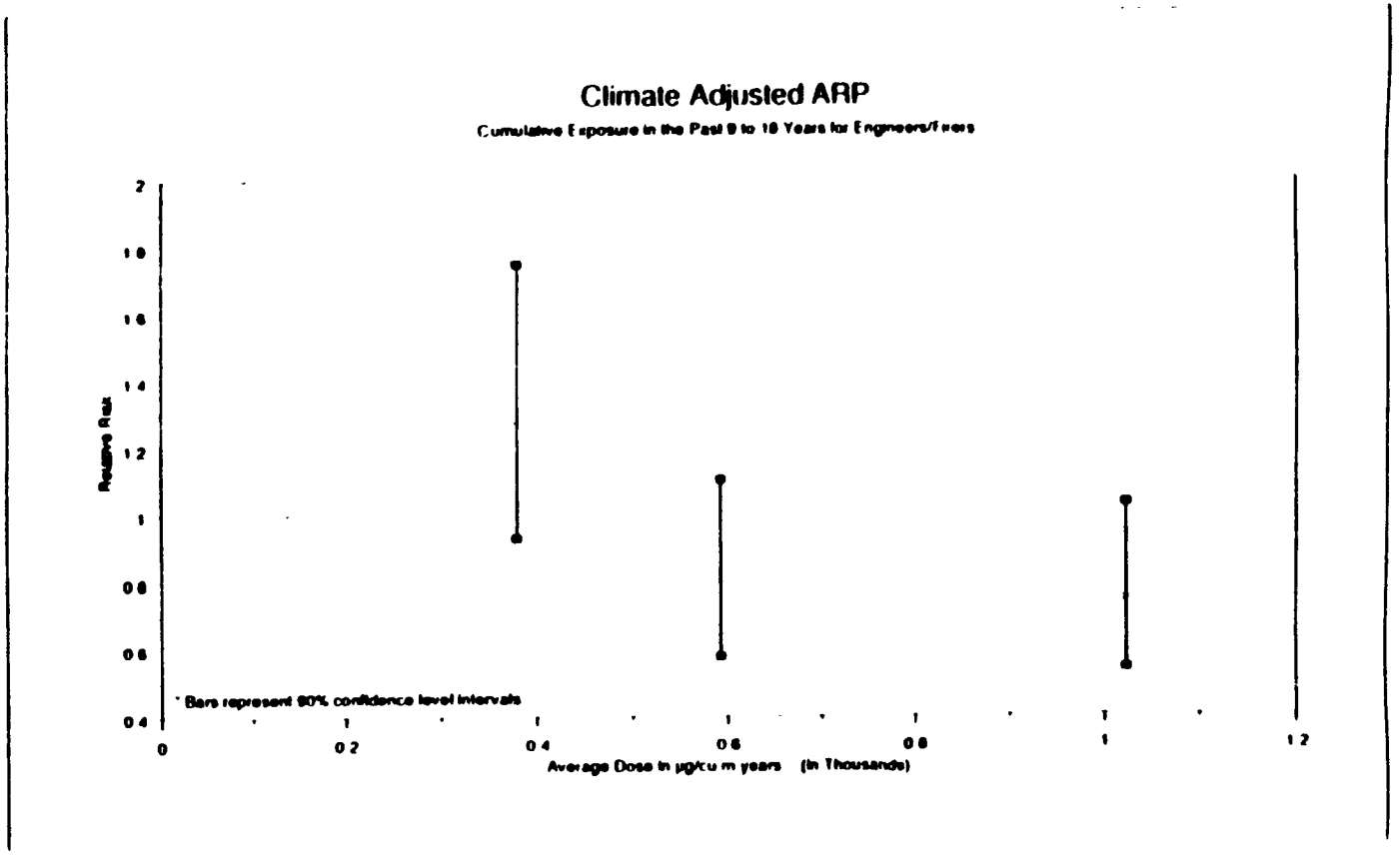
# Figure A2.4


Climate Adjusted ARP
Cumulative Exposure in the Past 4 to 8 Years for Shipyard Workers
Relative Risk
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

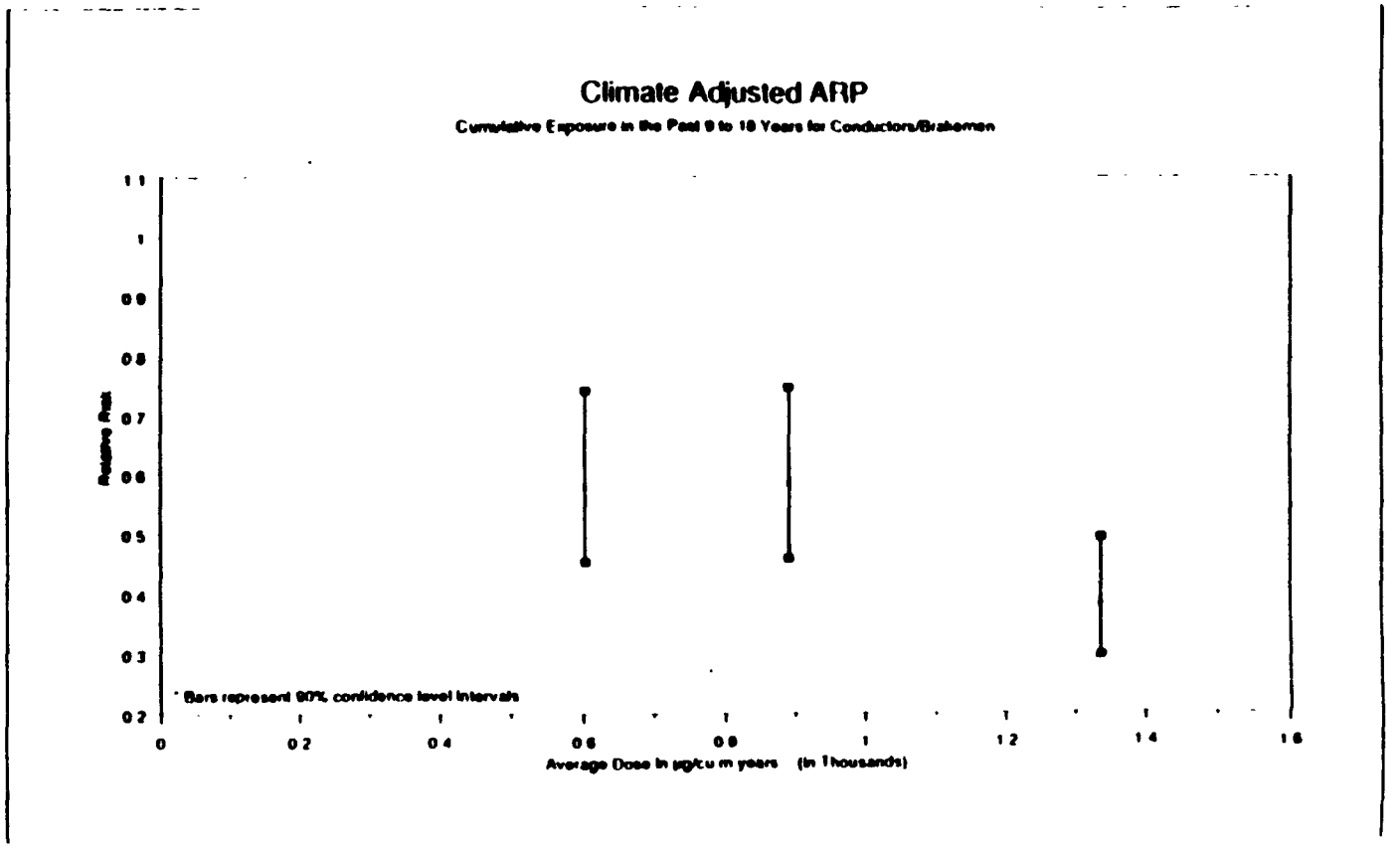
# Figure A2.5


Climate Adjusted ARP
Cumulative Exposure in the Past 9 to 18 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average Dose in µg/cu m years (in Thousands)

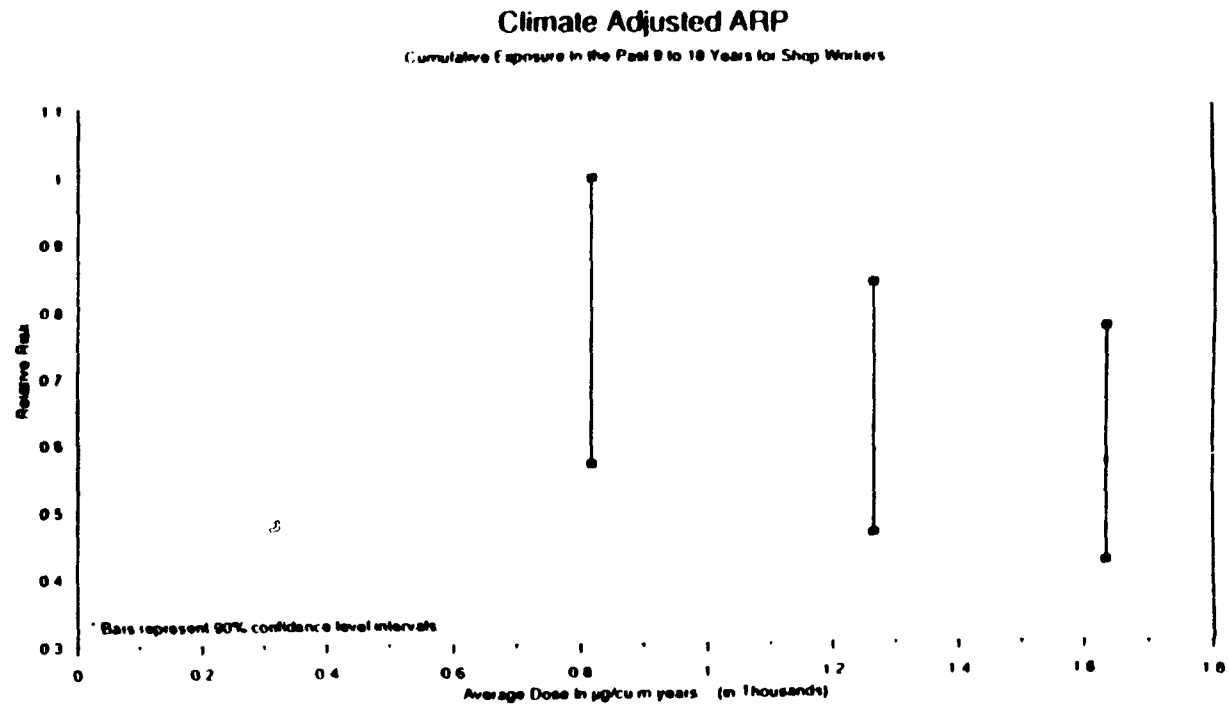
# Figure A2.6


Climate Adjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Engineers/Fires
Relative Risk
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

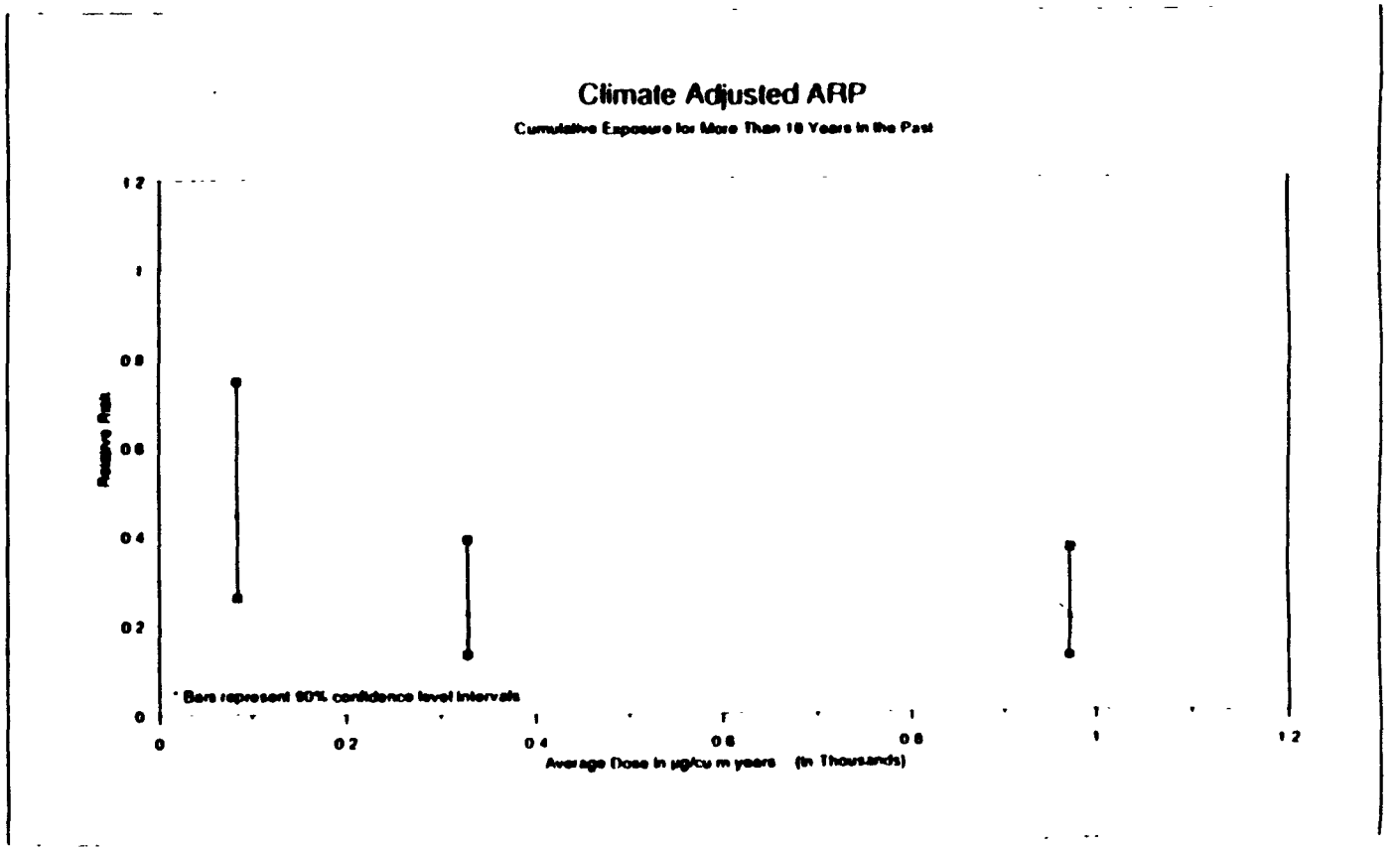
# Figure A2.7


Climate Adjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Average Dose in µg/cu m years (in Thousands)

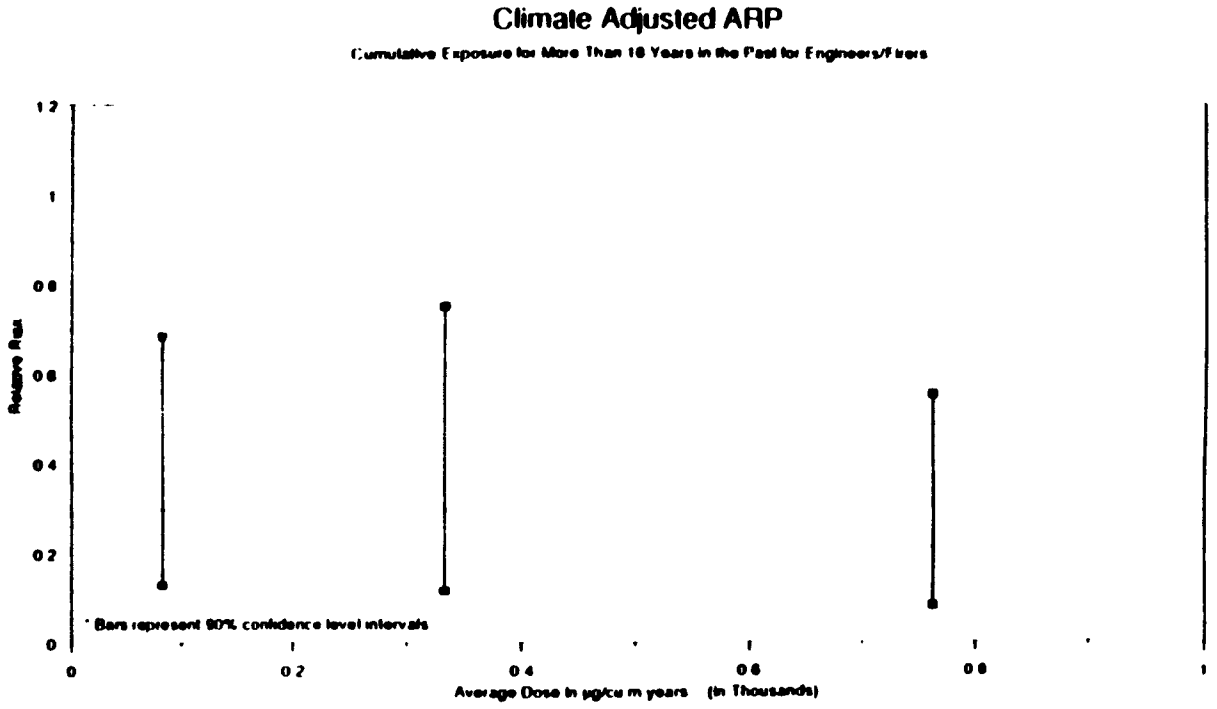
# Figure A2.8


Climate Adjusted ARP
Cumulative Exposure in the Past 9 to 18 Years for Shop Workers
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Dose in µg/cu m years (in Thousands)

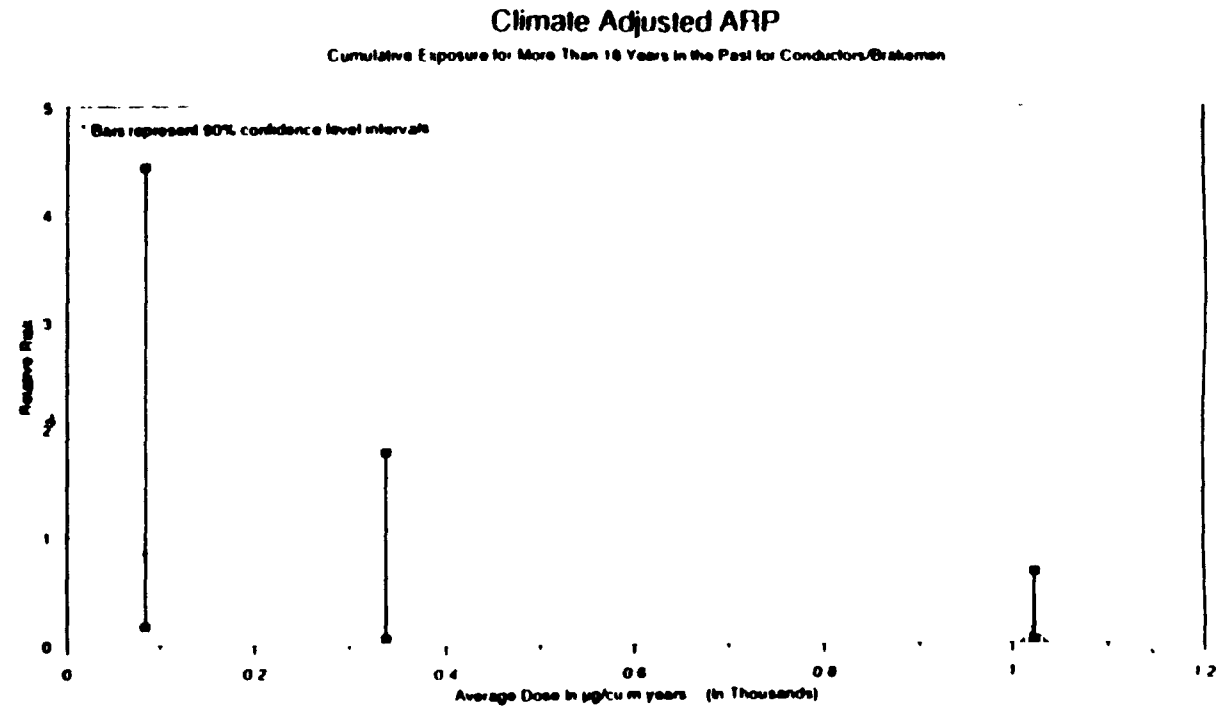
# Figure A2.9


Climate Adjusted ARP
Cumulative Exposure for More Than 10 Years in the Past
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

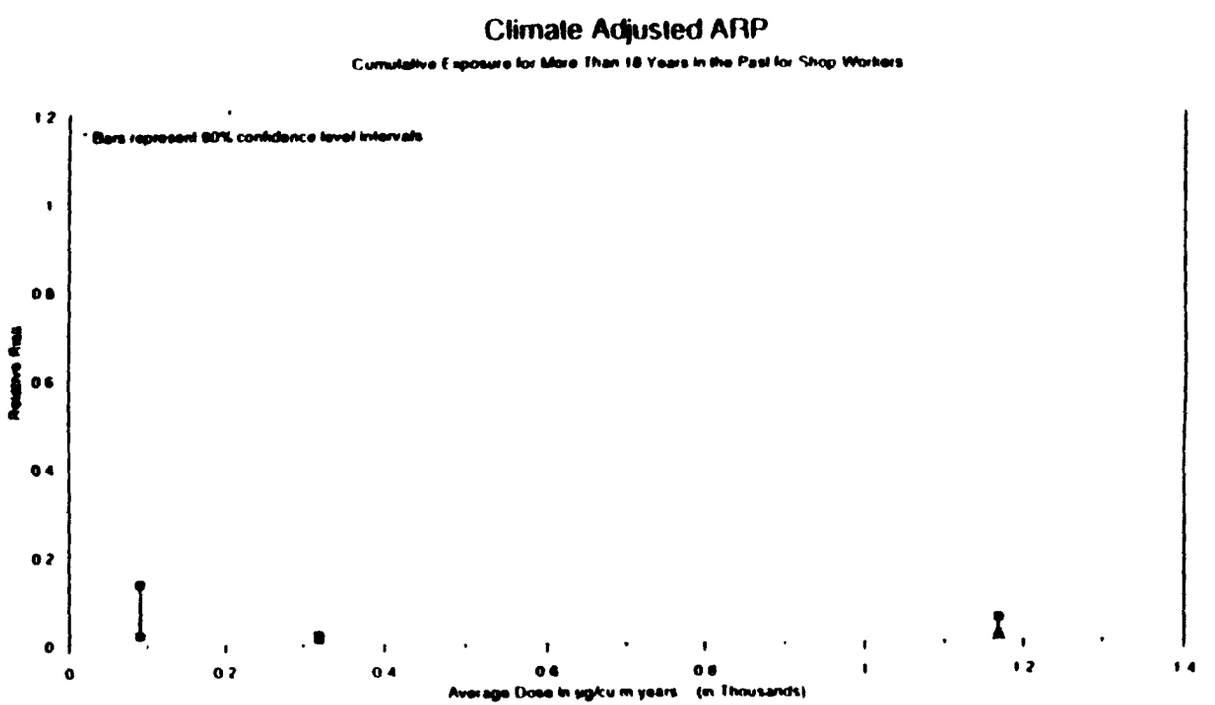
# Figure A2.10


Climate Adjusted ARP
Cumulative Exposure for More Than 10 Years in the Past for Engineers/Firers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

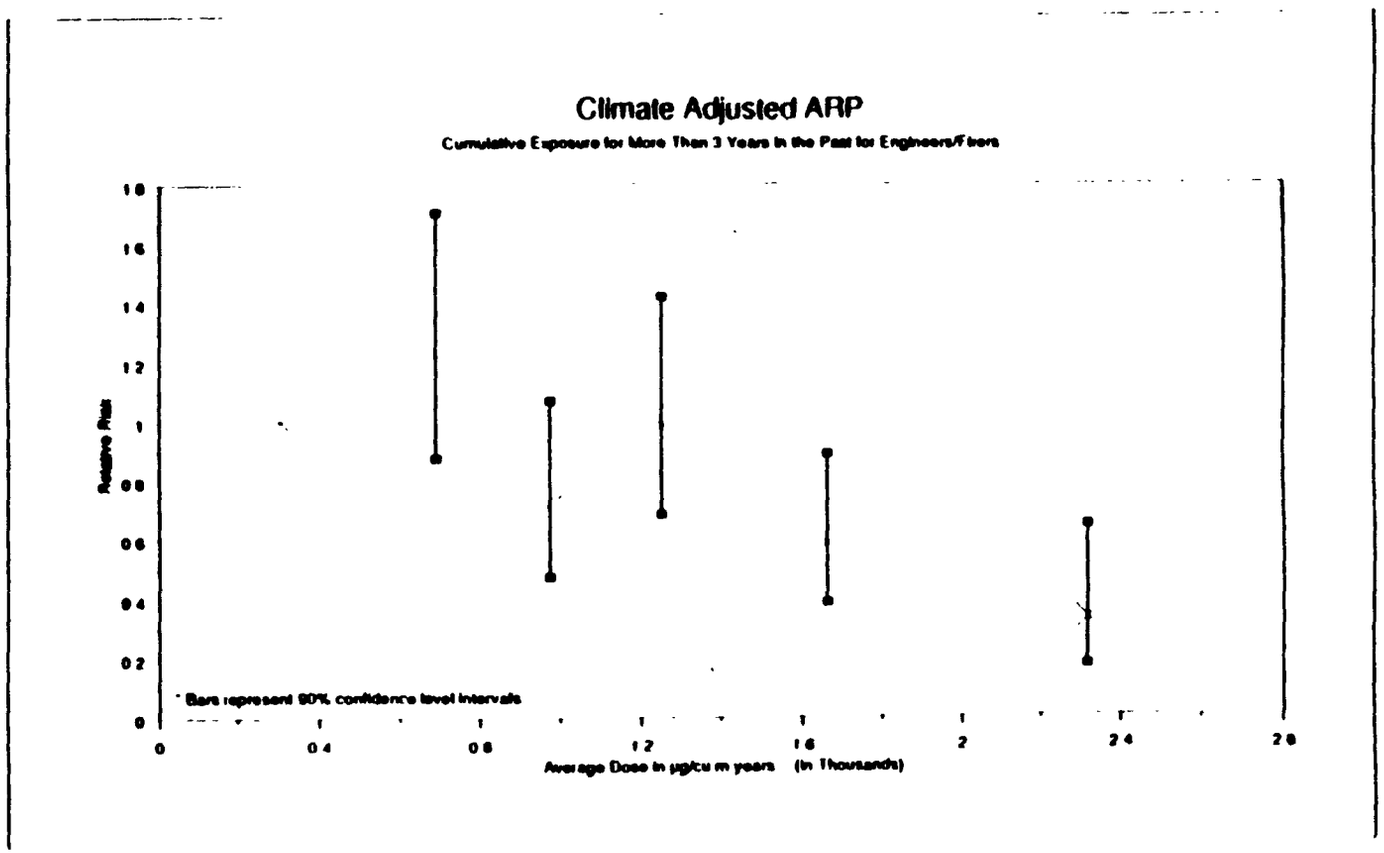
# Figure A2.11


Climate Adjusted ARP
Cumulative Exposure for More Than 10 Years in the Past for Conductors/Brakemen
* Bars represent 90% confidence level intervals
Relative Risk
0
1
2
3
4
5
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

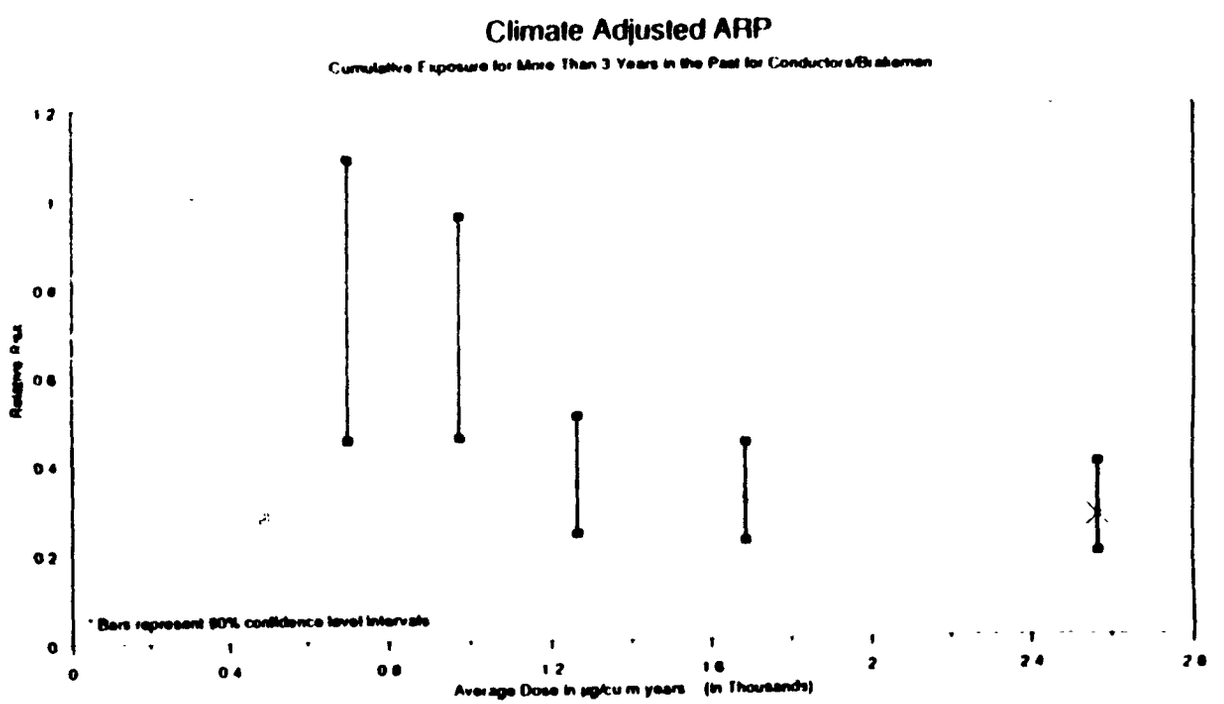
# Figure A2.12


Climate Adjusted ARP
Cumulative Exposure for More Than 18 Years in the Past for Shop Workers
* Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average Dose in sg/cu m years (in Thousands)

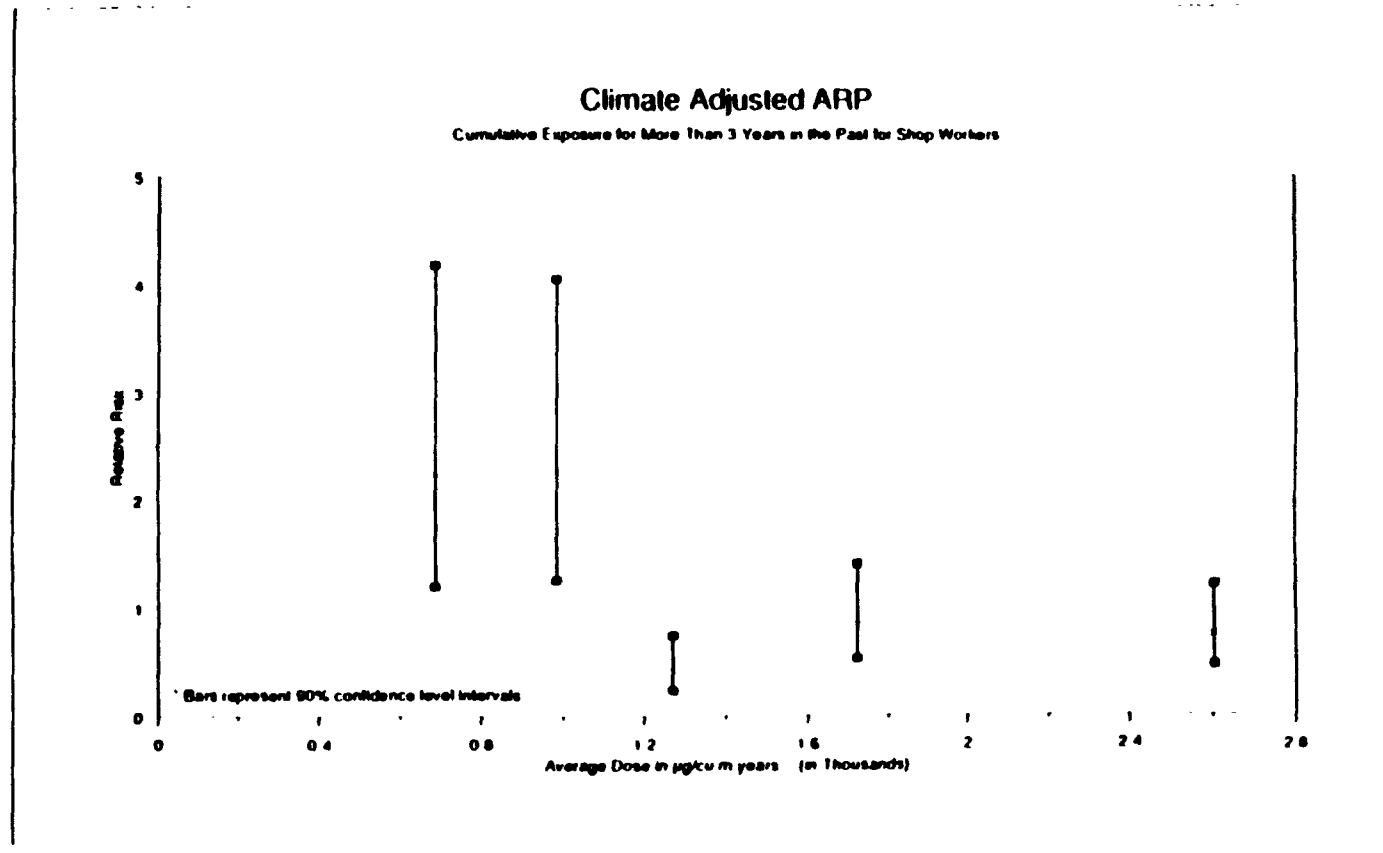
# Figure A2.13


Climate Adjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Engineers/Fibers
Relative Risk
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Average Dose in µg/cu m years (in Thousands)

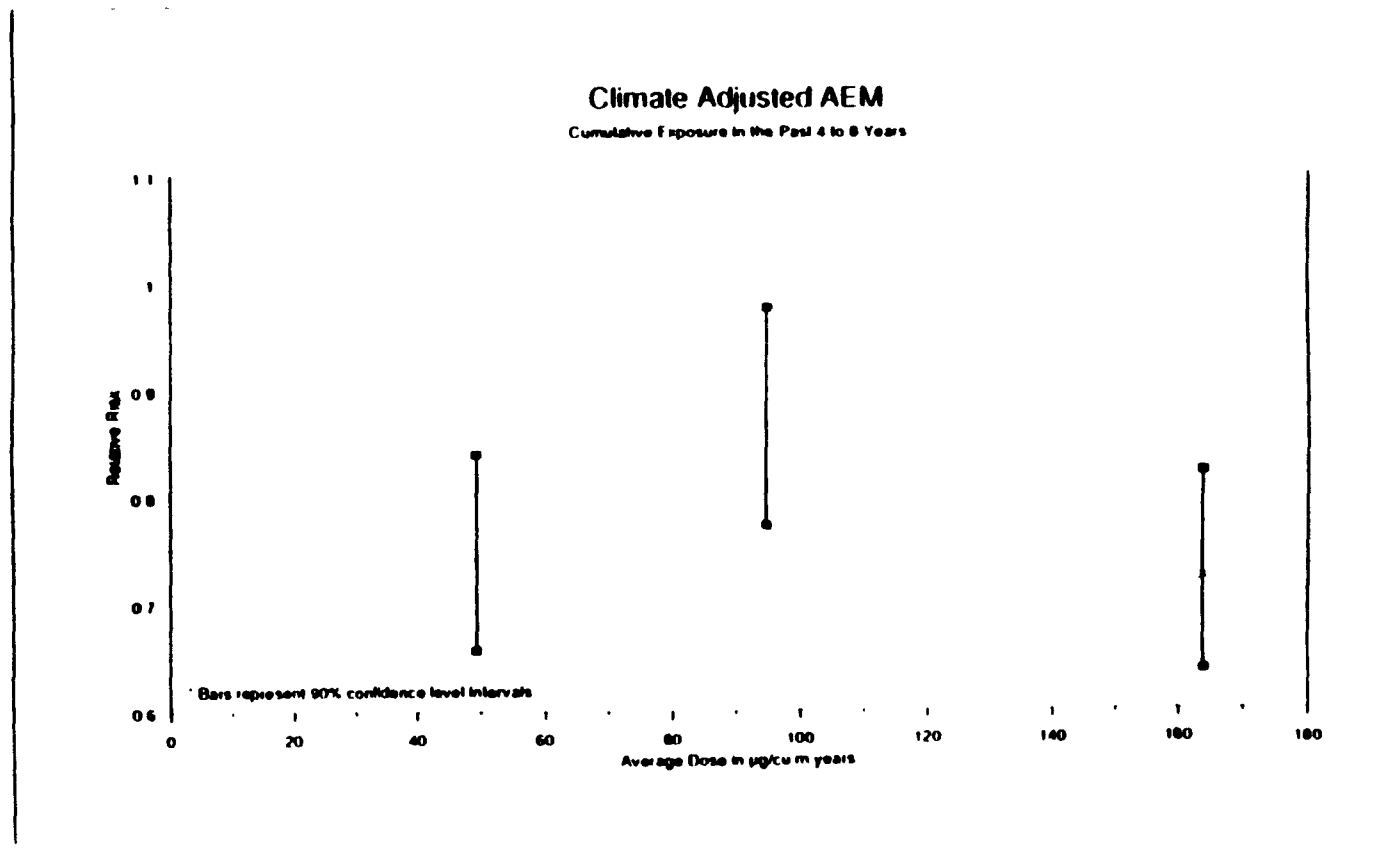
# Figure A2.14


Climate Adjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Conductors/Brakemen
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Average Dose in µg/cu m years (in Thousands)

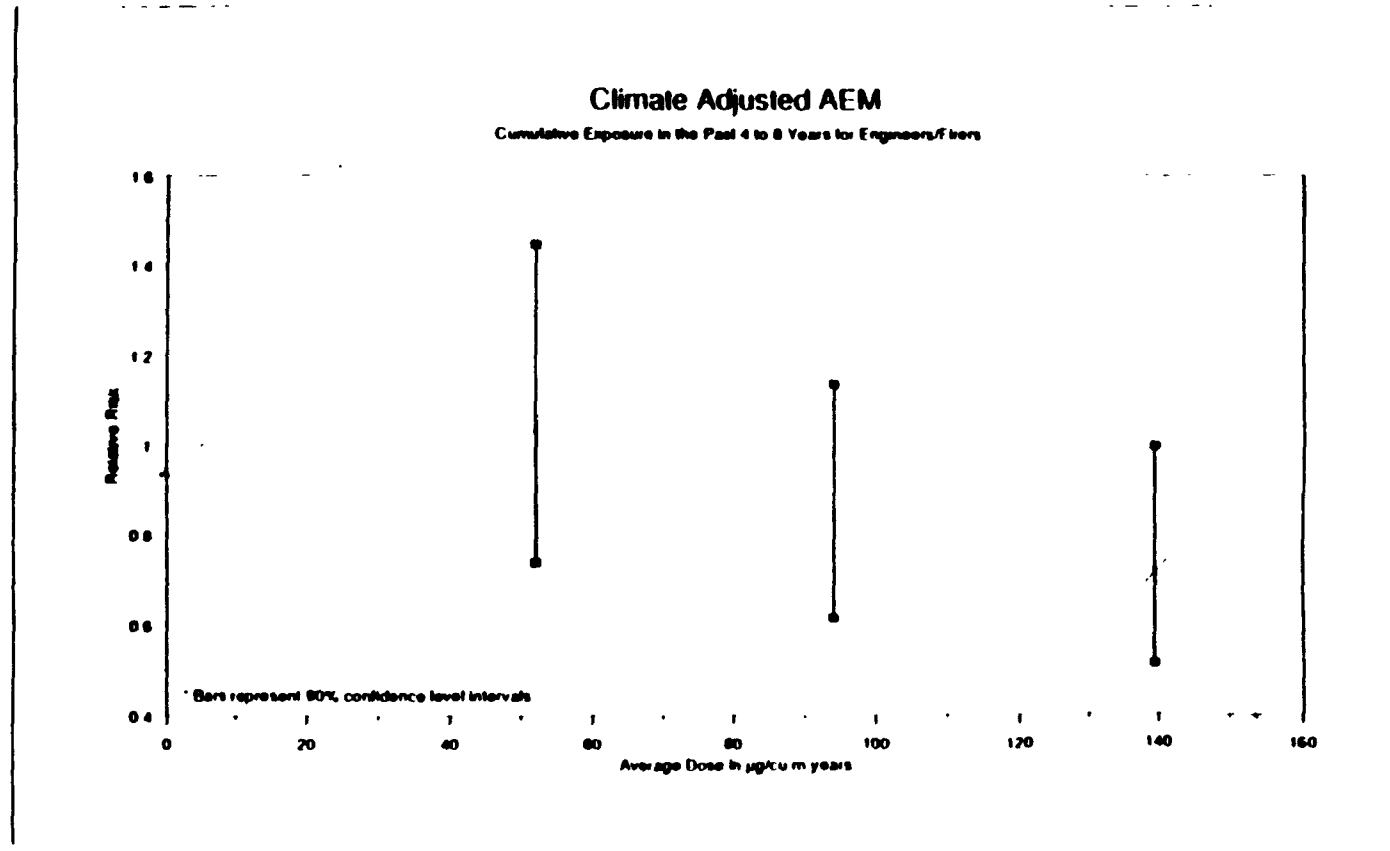
# Figure A2.15


Climate Adjusted ARP
Cumulative Exposure for More Than 3 Years in the Past for Shop Workers
Relative Risk
0
1
2
3
4
5
* Bars represent 90% confidence level intervals
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Average Dose in µg/cu m years (in Thousands)

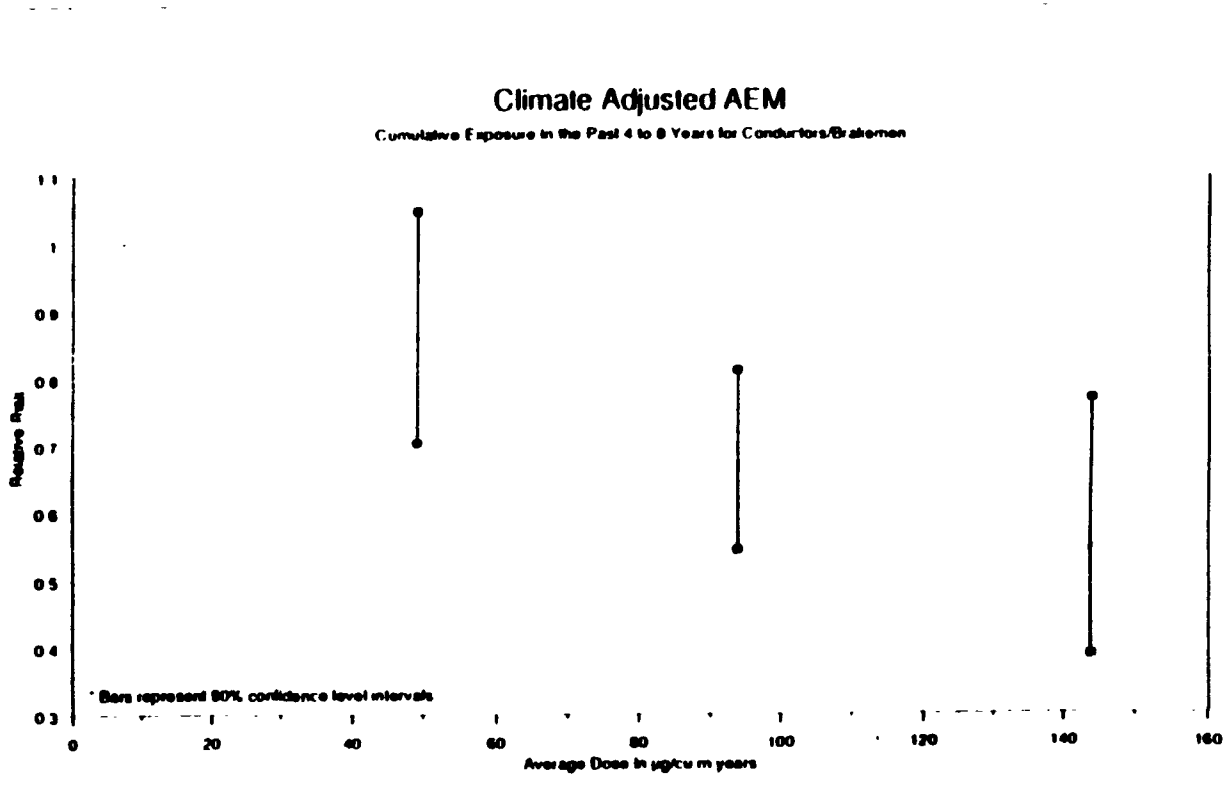
# Figure A3.1


Climate Adjusted AEM
Cumulative Exposure in the Past 4 to 8 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
Bars represent 90% confidence level intervals
0
20
40
60
80
100
120
140
160
180
Average Dose in µg/cu m years

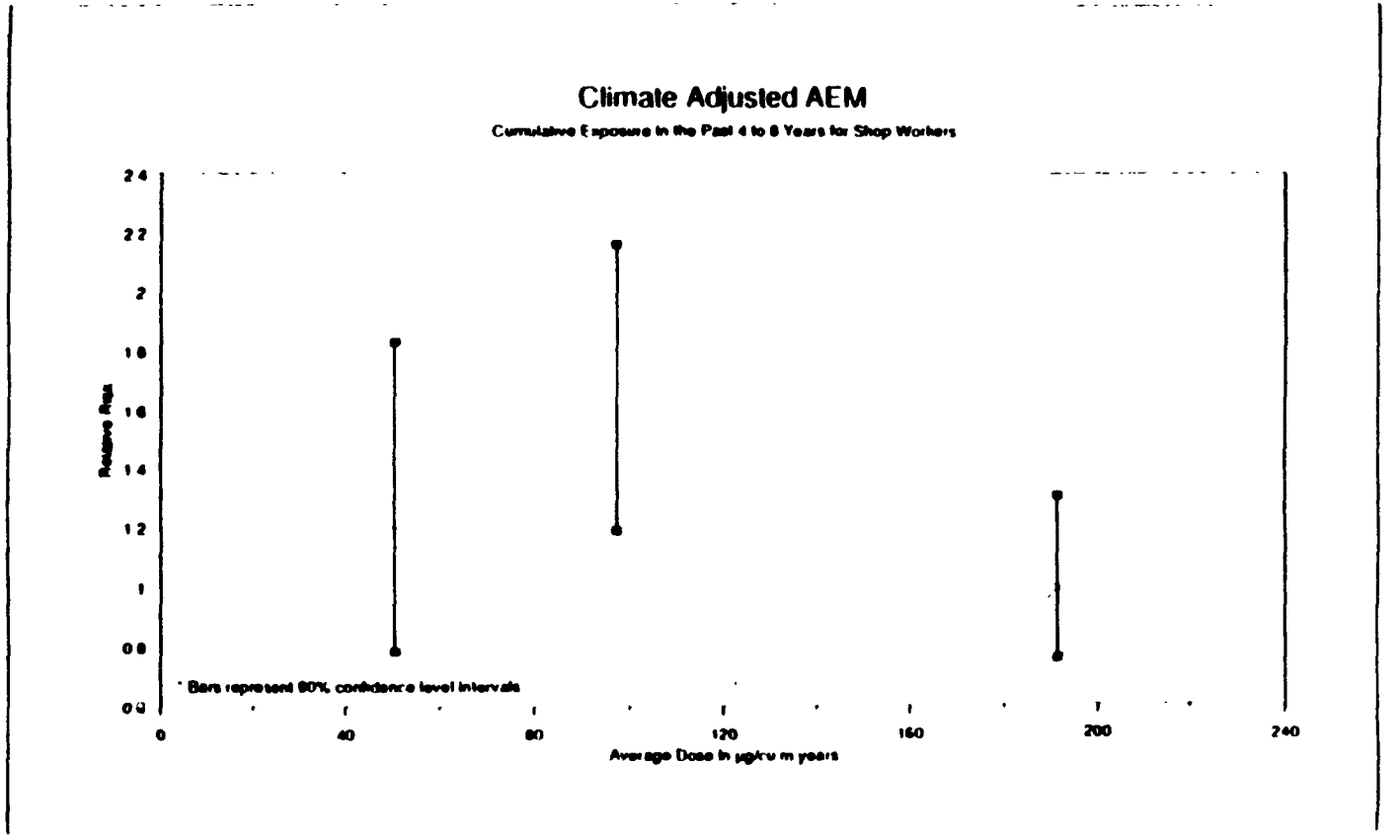
# Figure A3.2


Climate Adjusted AEM
Cumulative Exposure in the Past 4 to 8 Years for Engineers/Fitters
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
20
40
60
80
100
120
140
160
Average Dose in µg/cu m years

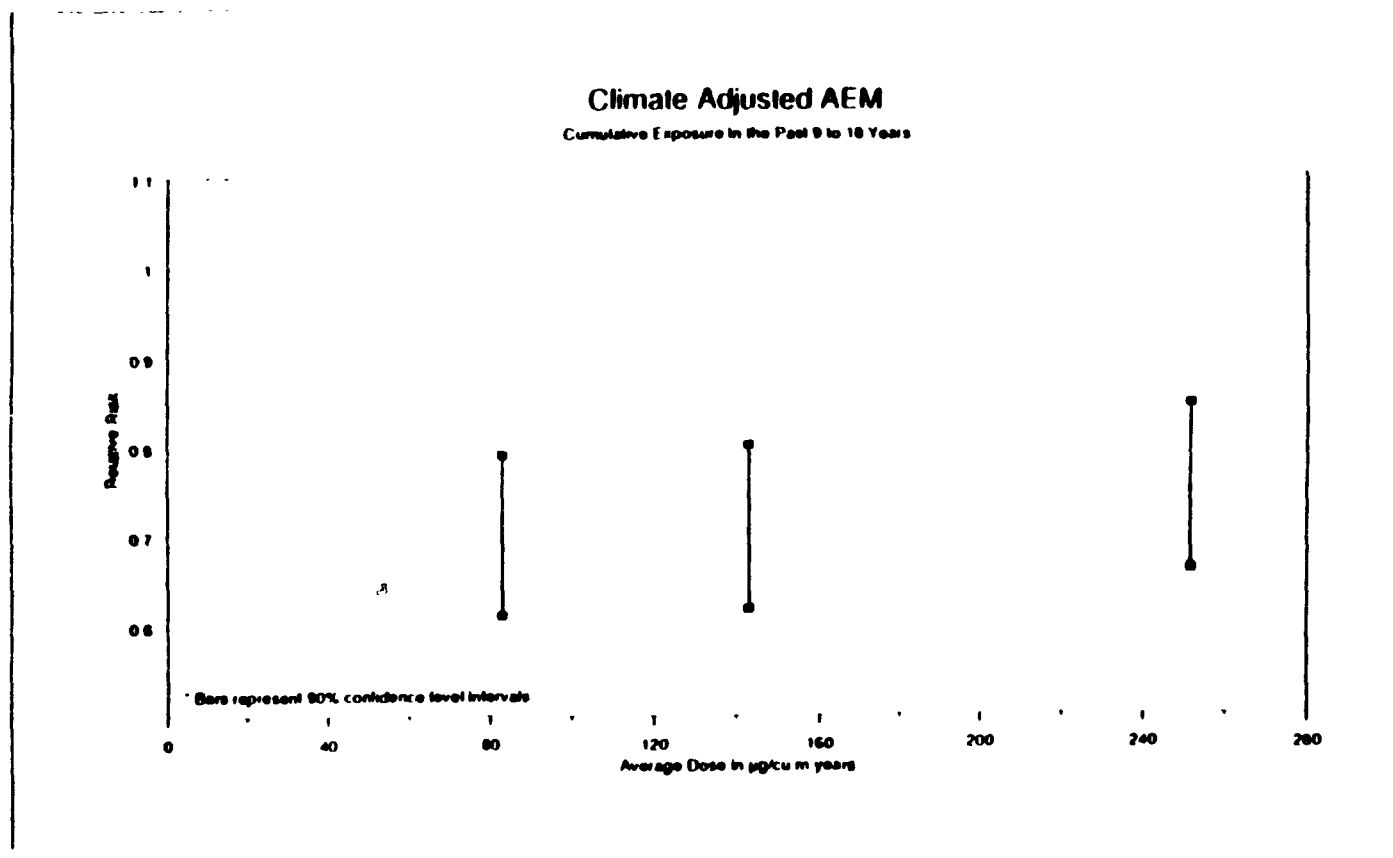
# Figure A3.3


Climate Adjusted AEM
Cumulative Exposure in the Past 4 to 8 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
20
40
60
80
100
120
140
160
Average Dose in µg/cu m years

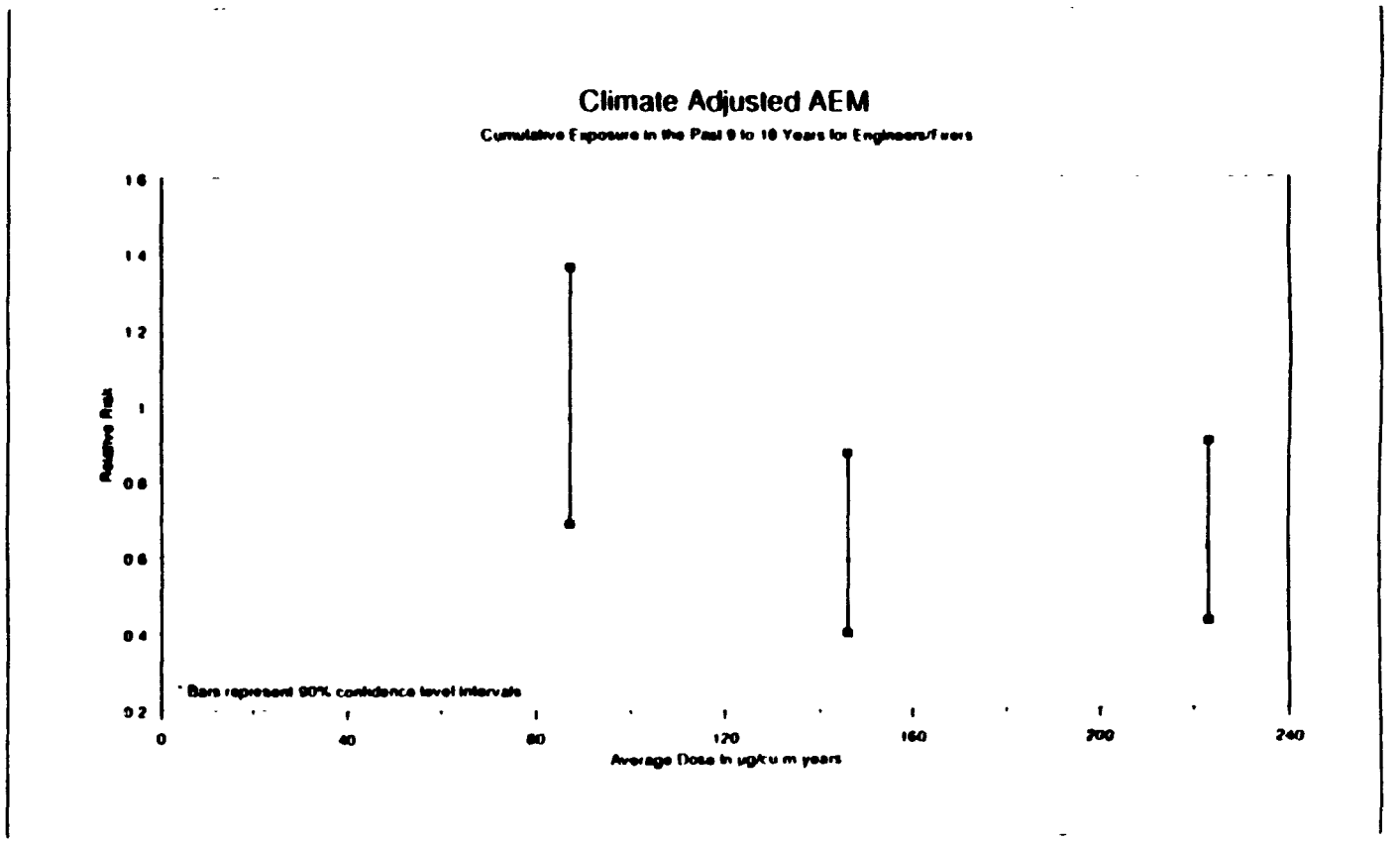
# Figure A3.4


Climate Adjusted AEM
Cumulative Exposure in the Past 4 to 8 Years for Shop Workers
Relative Risk
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
Average Dose in µg/cu m years

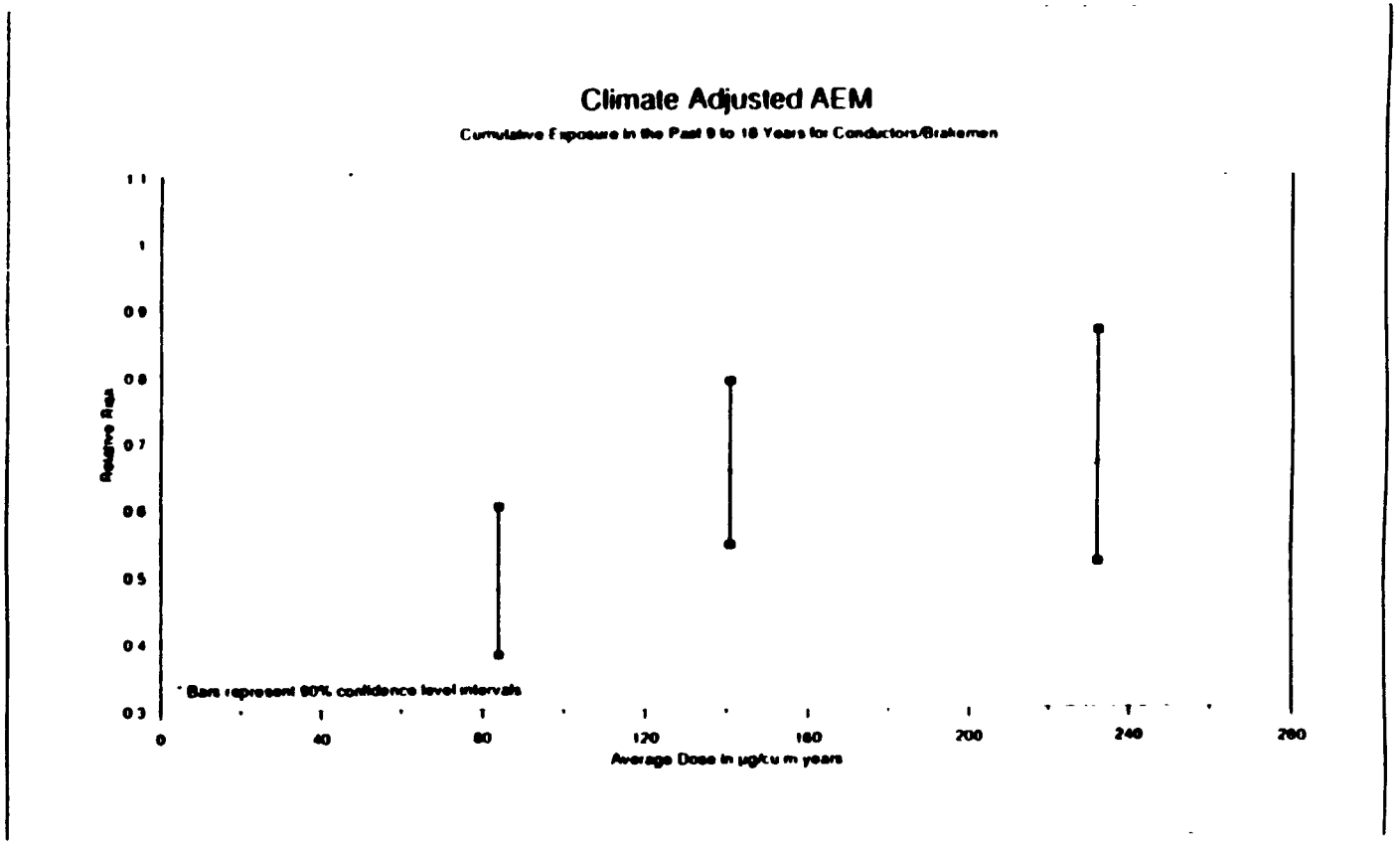
# Figure A3.5


Climate Adjusted AEM
Cumulative Exposure in the Past 9 to 18 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
280
Average Dose in µg/cu m years

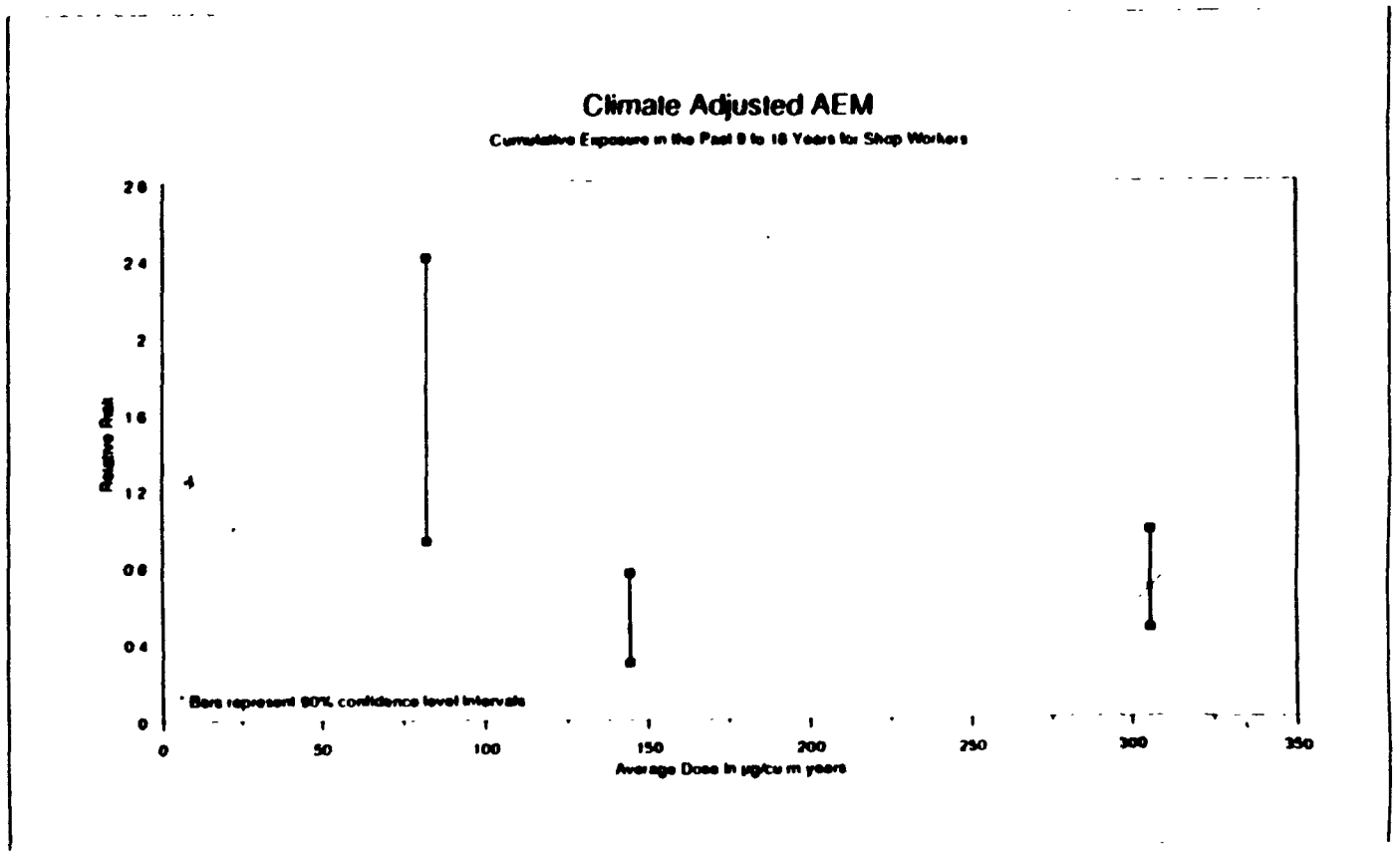
# Figure A3.6


Climate Adjusted AEM
Cumulative Exposure in the Past 9 to 18 Years for Engineers/Fivers
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
Average Dose in ug/cu m years

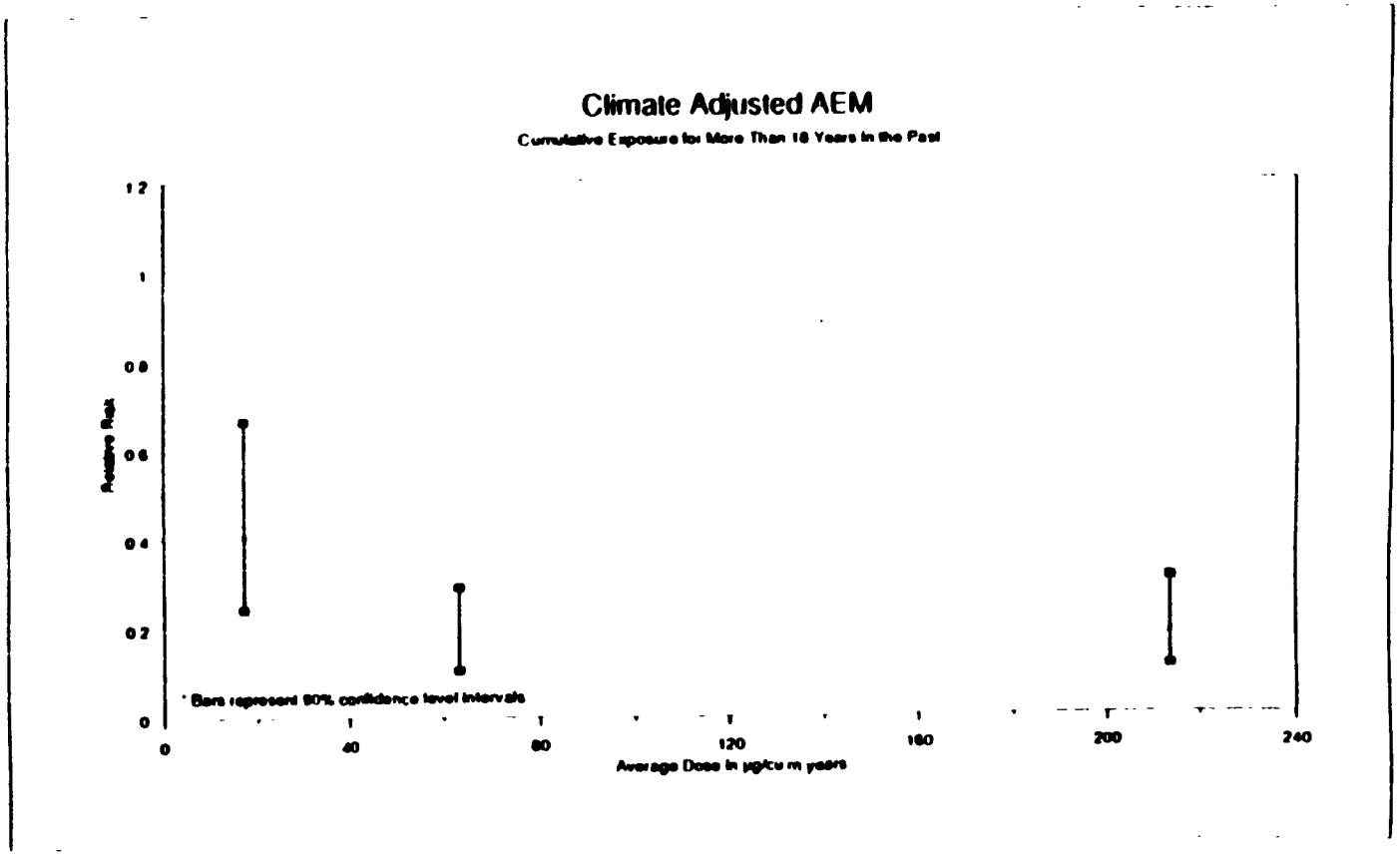
# Figure A3.7


Climate Adjusted AEM
Cumulative Exposure in the Past 9 to 18 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
280
Average Dose in µg/cu m years

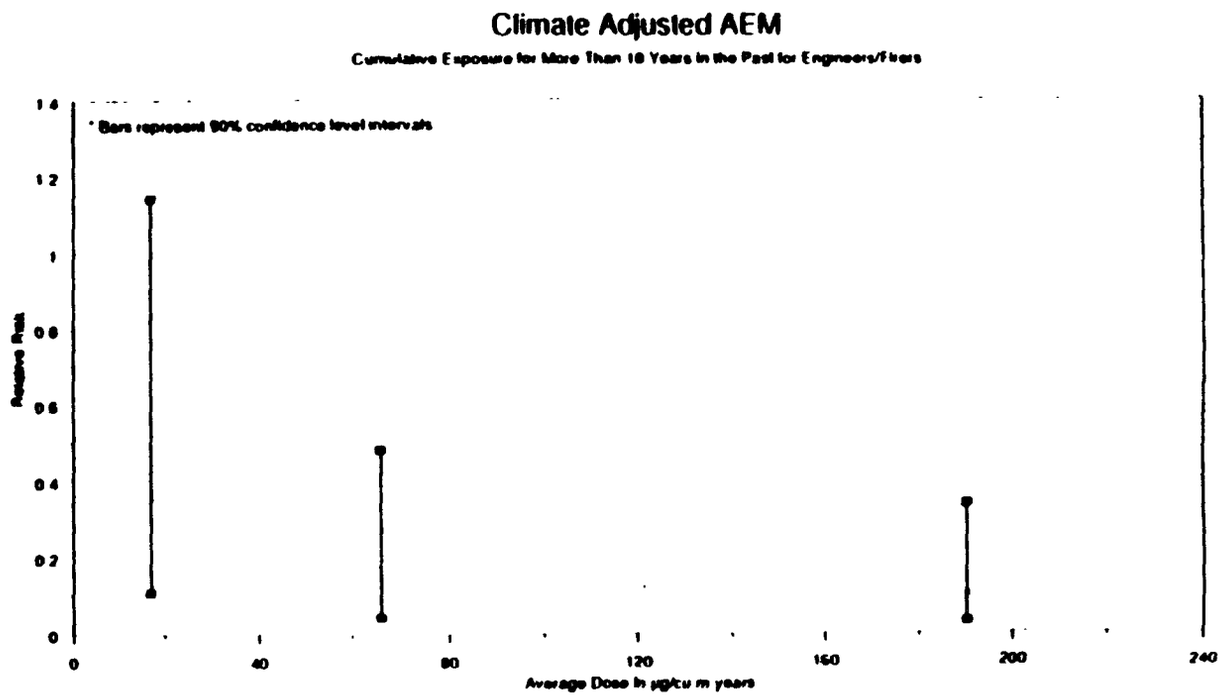
# Figure A3.8


Climate Adjusted AEM
Cumulative Exposure in the Past 9 to 18 Years for Shop Workers
Relative Risk
2.8
2.4
2
1.6
1.2
0.8
0.4
0
* Bars represent 90% confidence level intervals
0
50
100
150
200
250
300
350
Average Dose in µg/cu m years

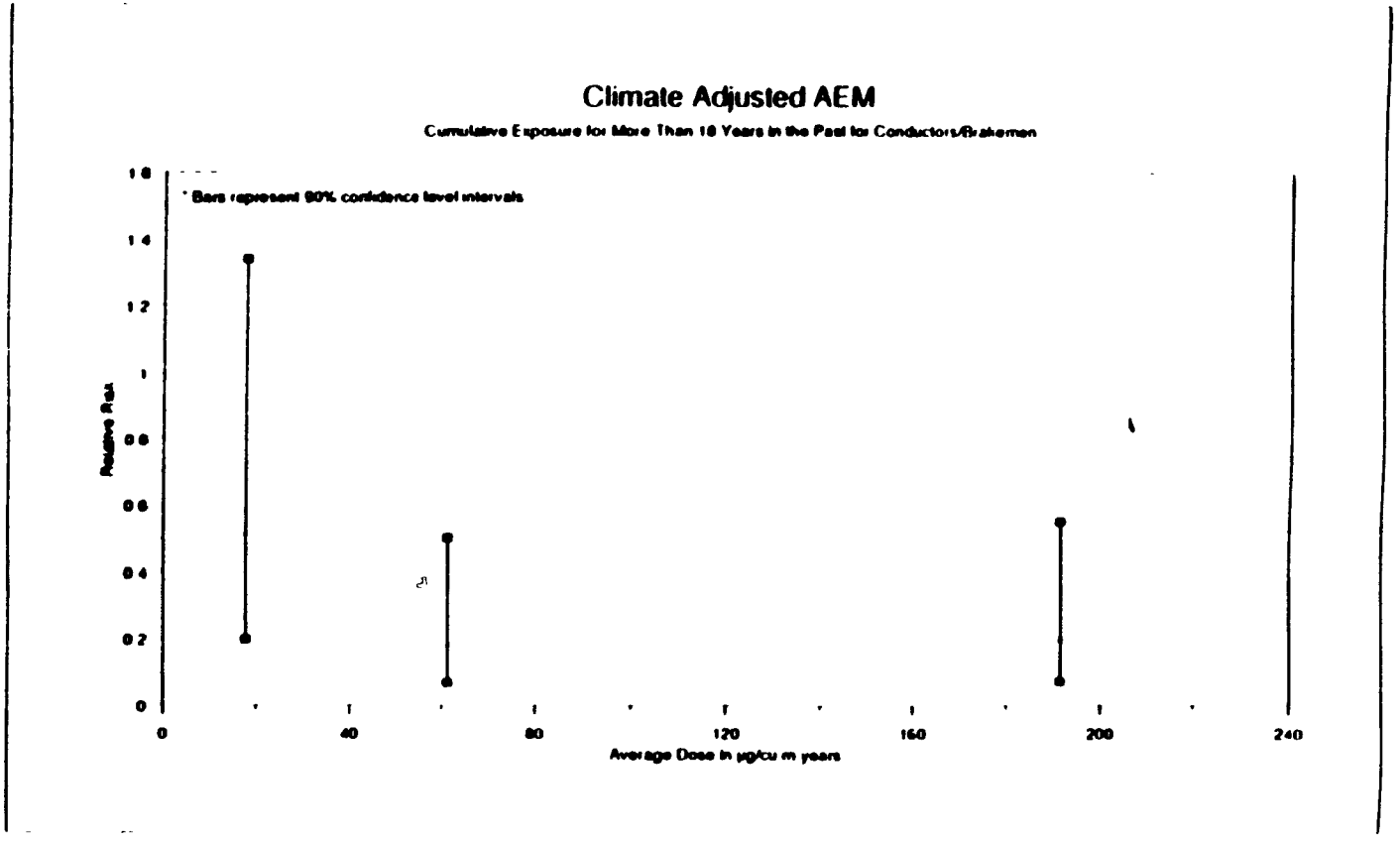
# Figure A3.9


Climate Adjusted AEM
Cumulative Exposure for More Than 18 Years in the Past
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
Average Dose in µg/cu m years

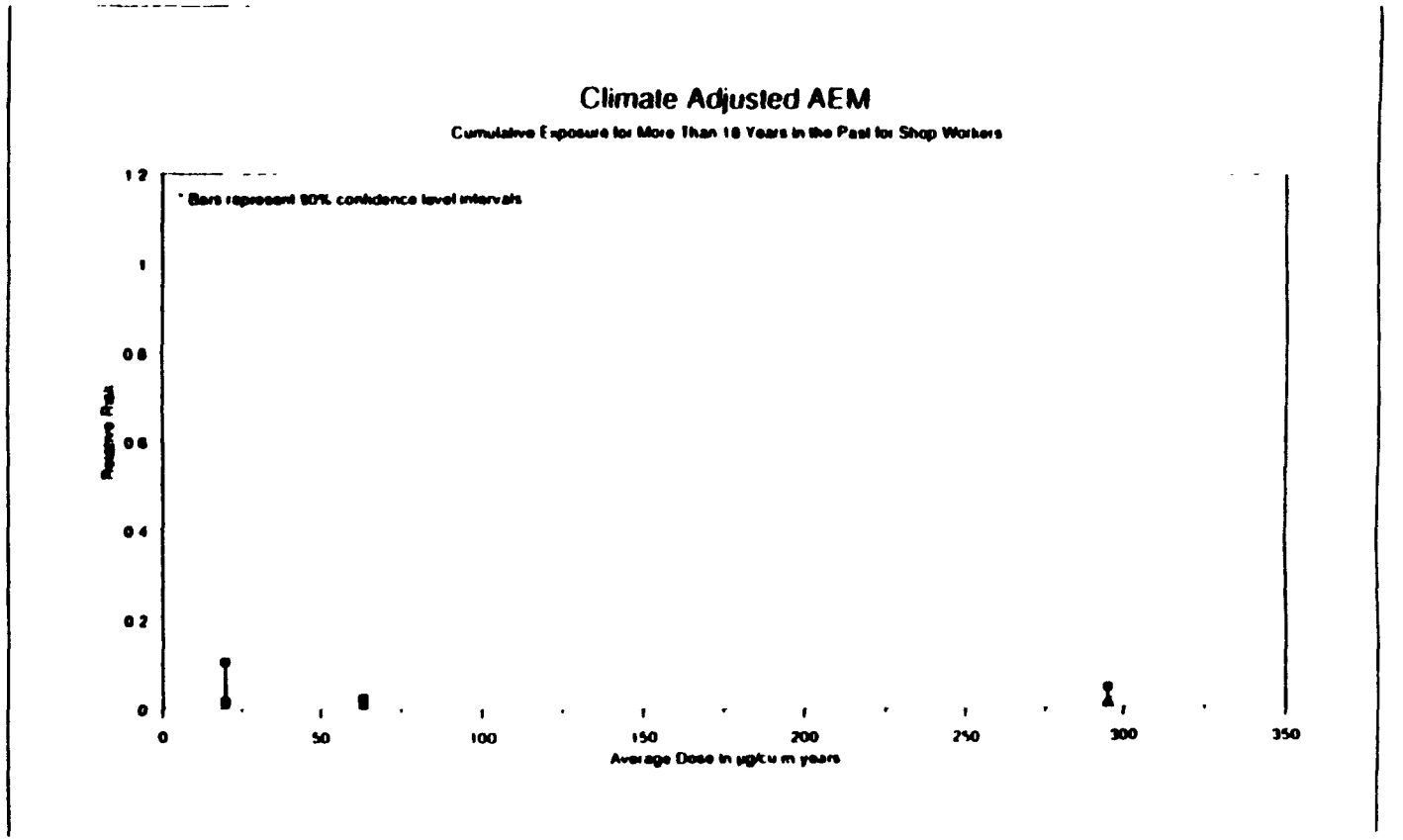
# Figure A3.10


Climate Adjusted AEM
Cumulative Exposure for More Than 10 Years in the Past for Engineers/Fixers
* Bars represent 90% confidence level intervals
Relative Risk
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
40
80
120
160
200
240
Average Dose in µg/cu m years

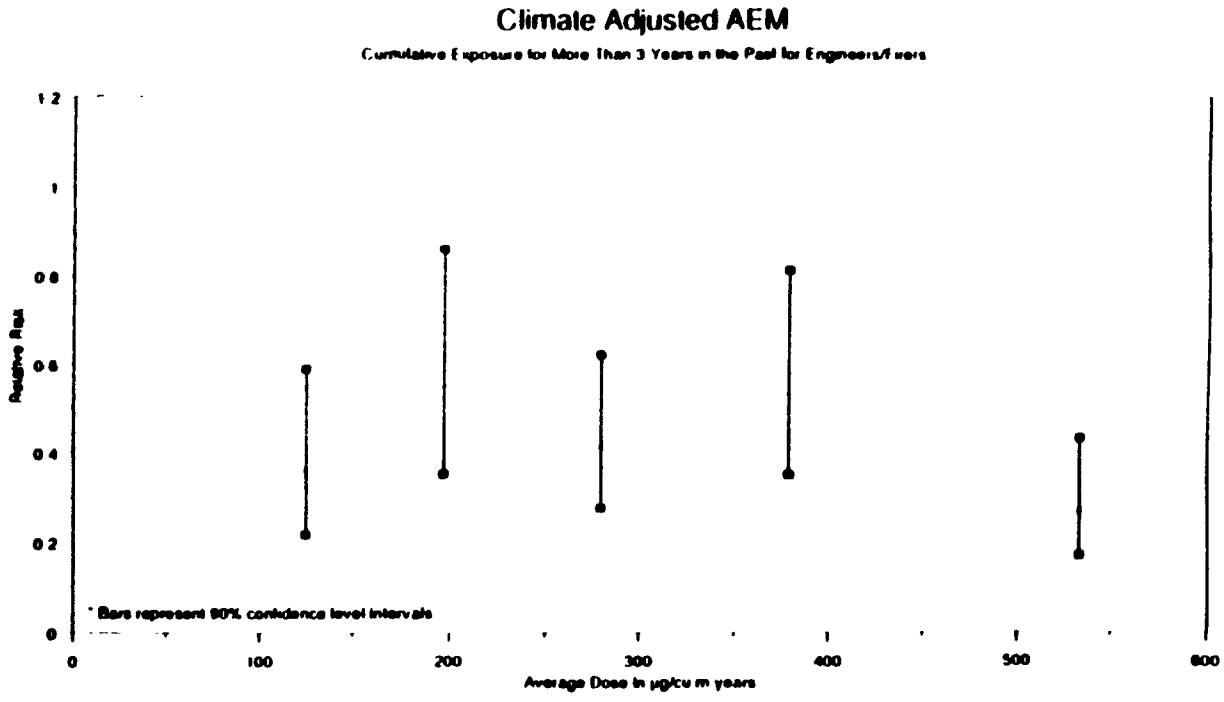
# Figure A3.11


Climate Adjusted AEM
Cumulative Exposure for More Than 10 Years in the Past for Conductors/Brakemen
* Bars represent 90% confidence level intervals
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
40
80
120
160
200
240
Average Dose in µg/cu m years

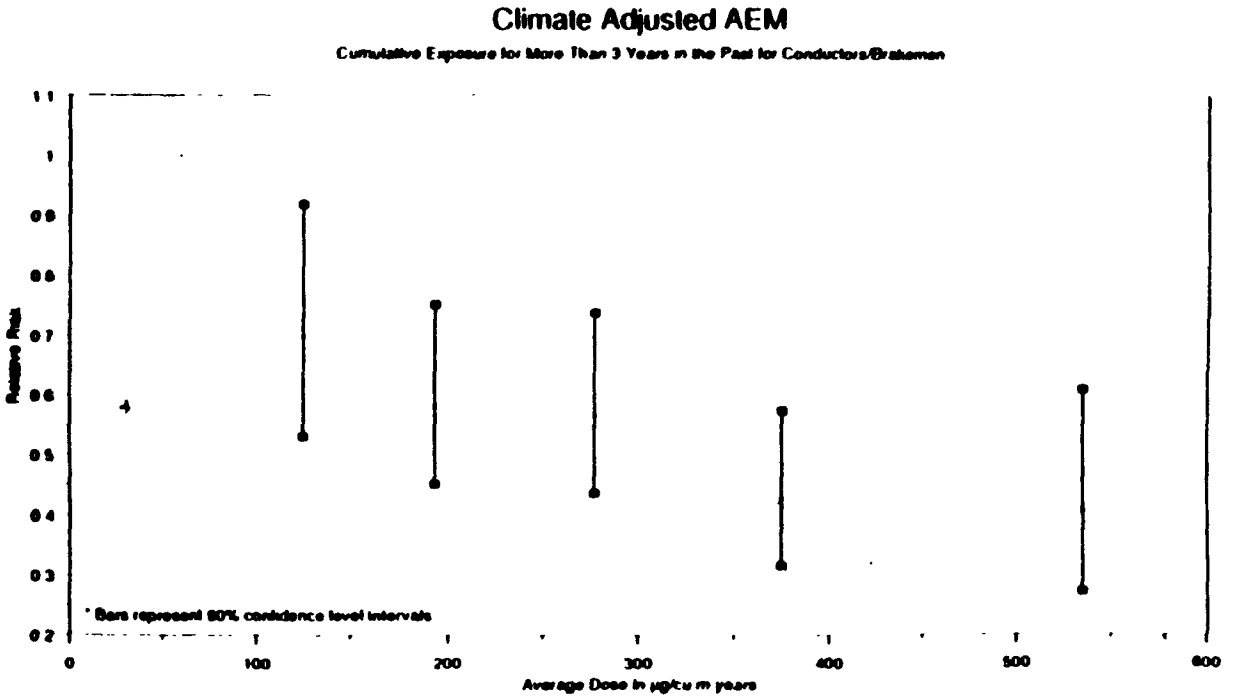
# Figure A3.12


Climate Adjusted AEM
Cumulative Exposure for More Than 18 Years in the Past for Shop Workers
* Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
350
Average Dose in µg/cu m years

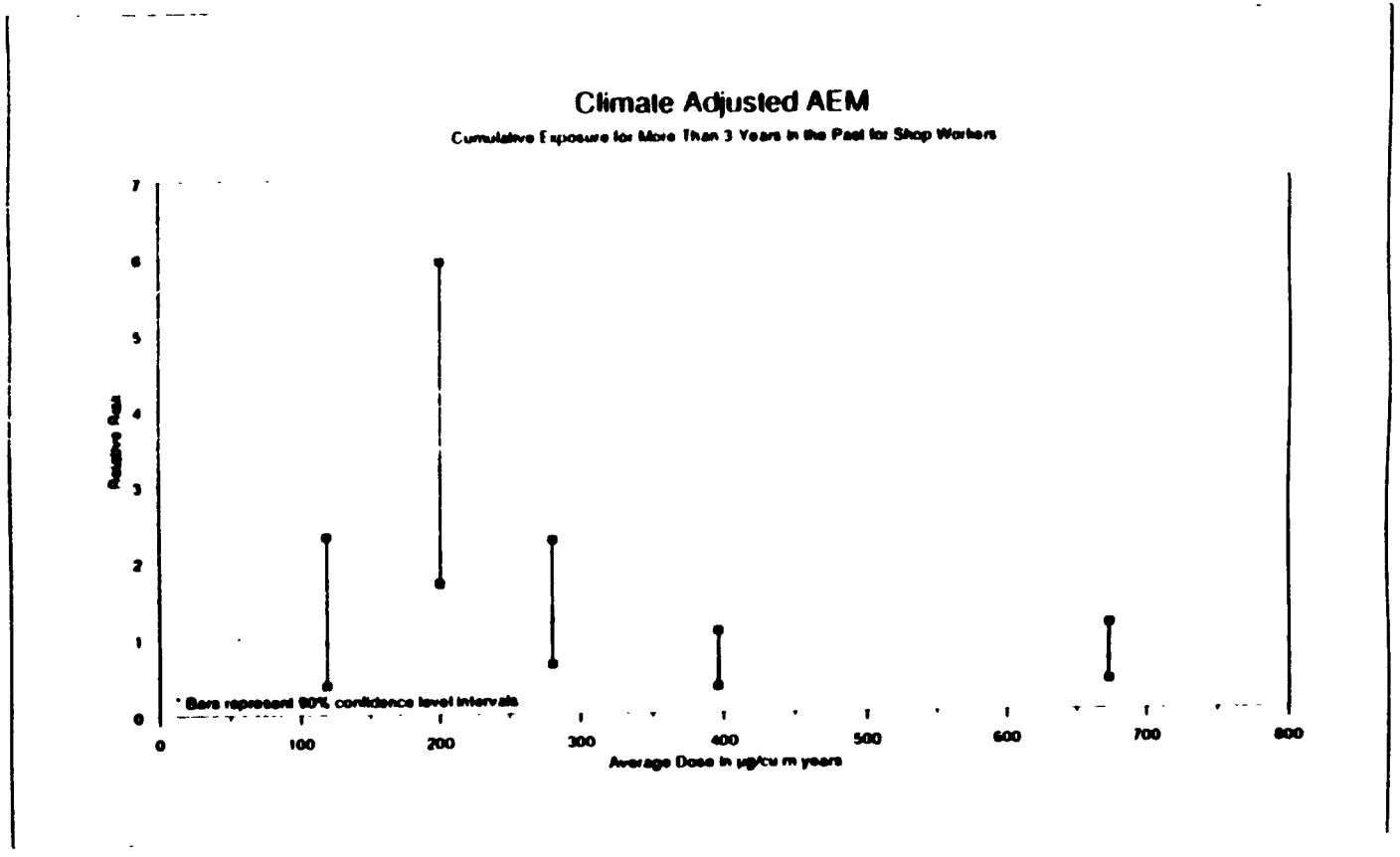
# Figure A3.13


Climate Adjusted AEM
Cumulative Exposure for More Than 3 Years in the Past for Engineers/Fivers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
Average Dose in µg/cu m years

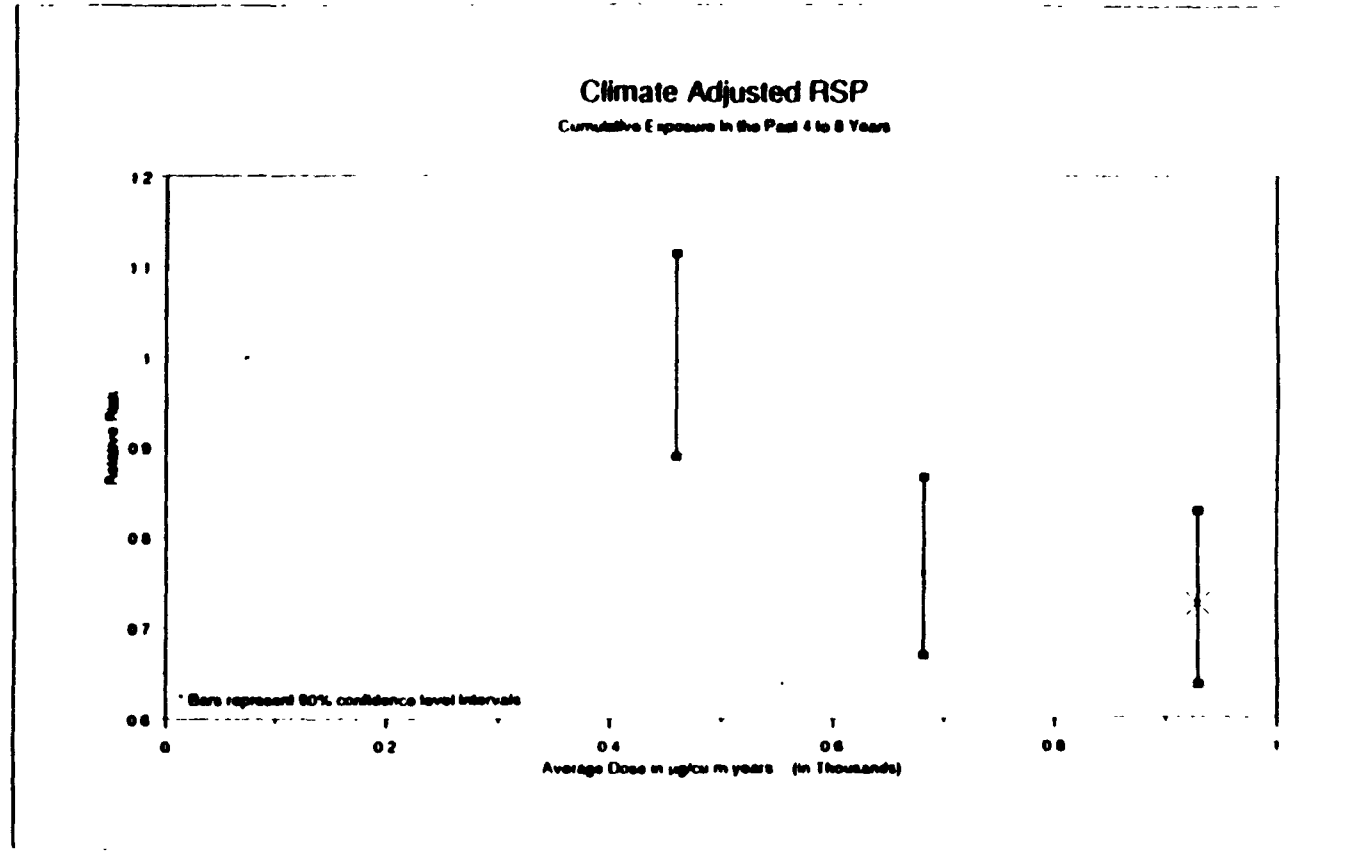
# Figure A3.14


Climate Adjusted AEM
Cumulative Exposure for More Than 3 Years in the Past for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
Average Dose in µg/cu m years

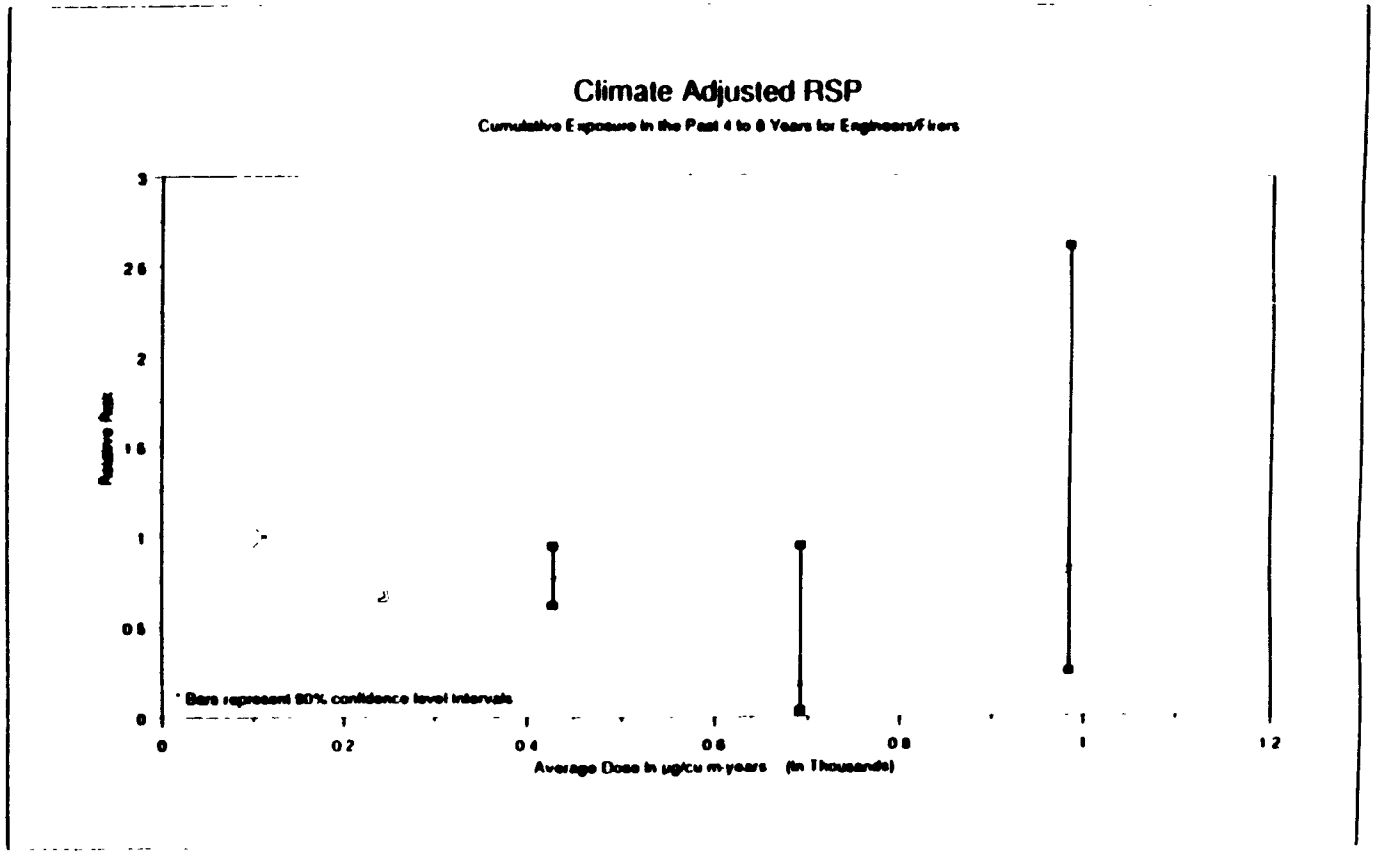
# Figure A3.15


Climate Adjusted AEM
Cumulative Exposure for More Than 3 Years in the Past for Shop Workers
Relative Risk
0
1
2
3
4
5
6
7
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
800
Average Dose in µg/cu m years

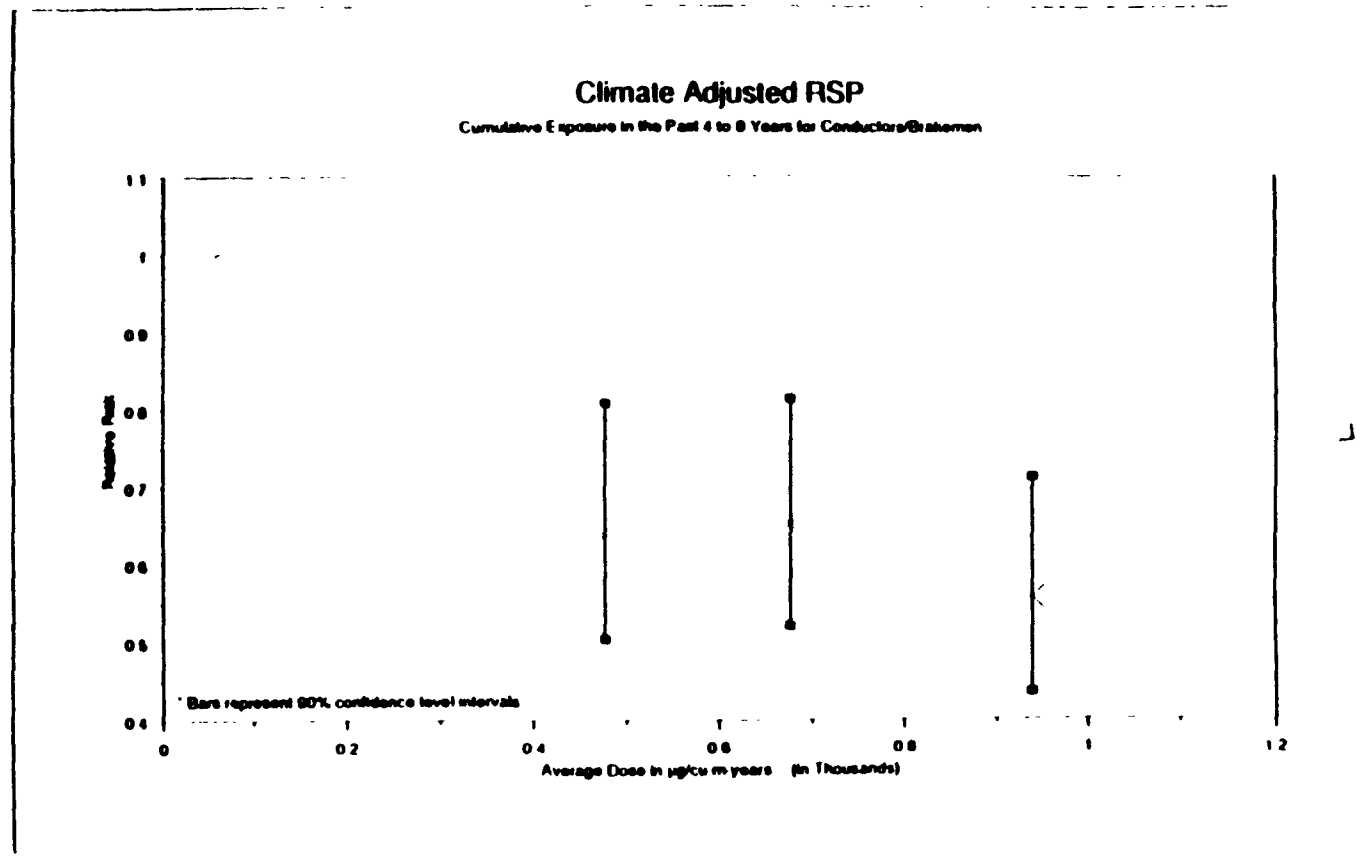
# Figure A4.1


Climate Adjusted RSP
Cumulative Exposure in the Past 4 to 8 Years
1.2
1.1
1
0.9
0.8
0.7
0.6
Relative Risk
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

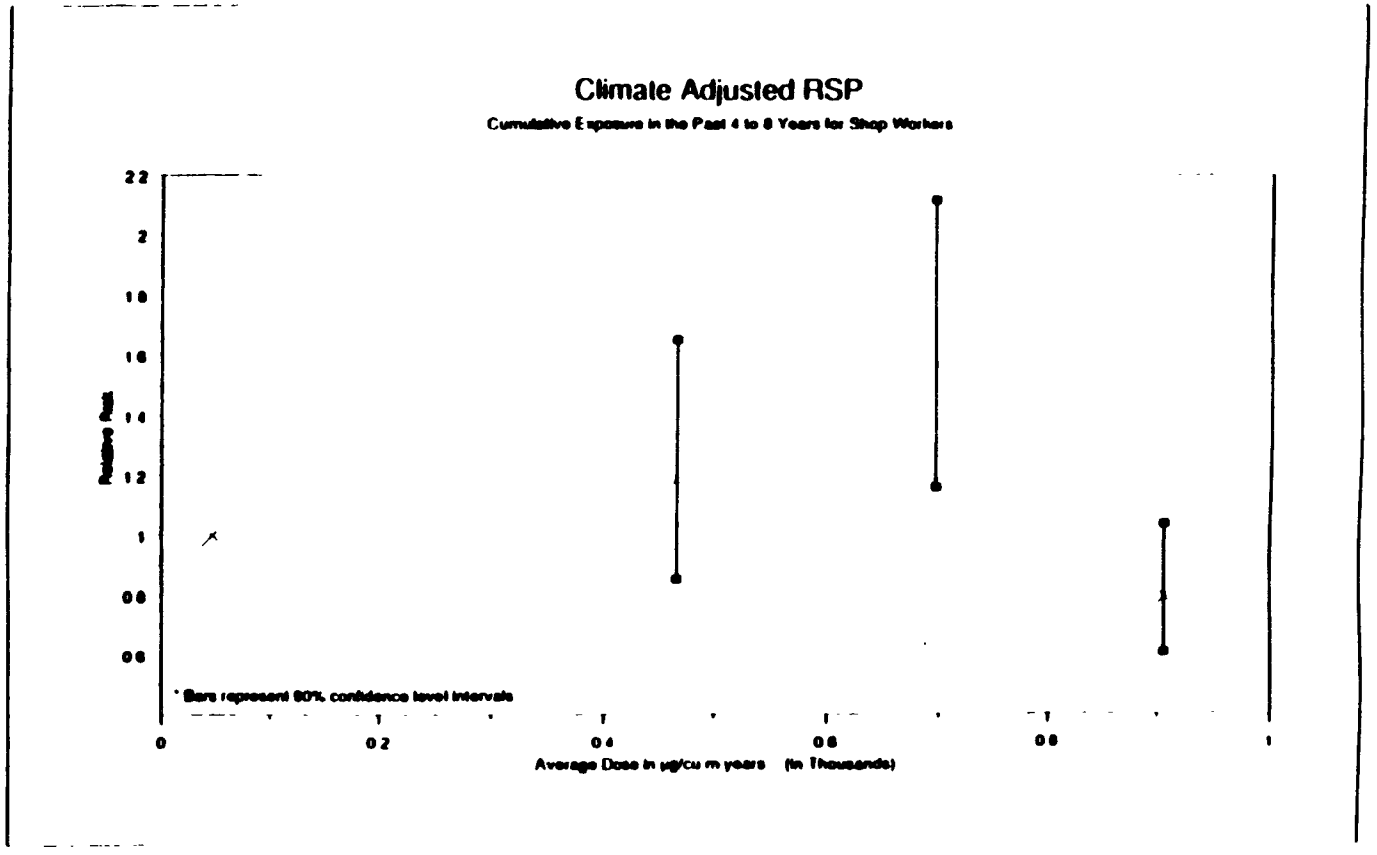
# Figure A4.2


Climate Adjusted RSP
Cumulative Exposure in the Past 4 to 8 Years for Engineers/Firers
3
2.5
2
1.5
1
0.5
0
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m-years (in Thousands)
* Bars represent 90% confidence level intervals

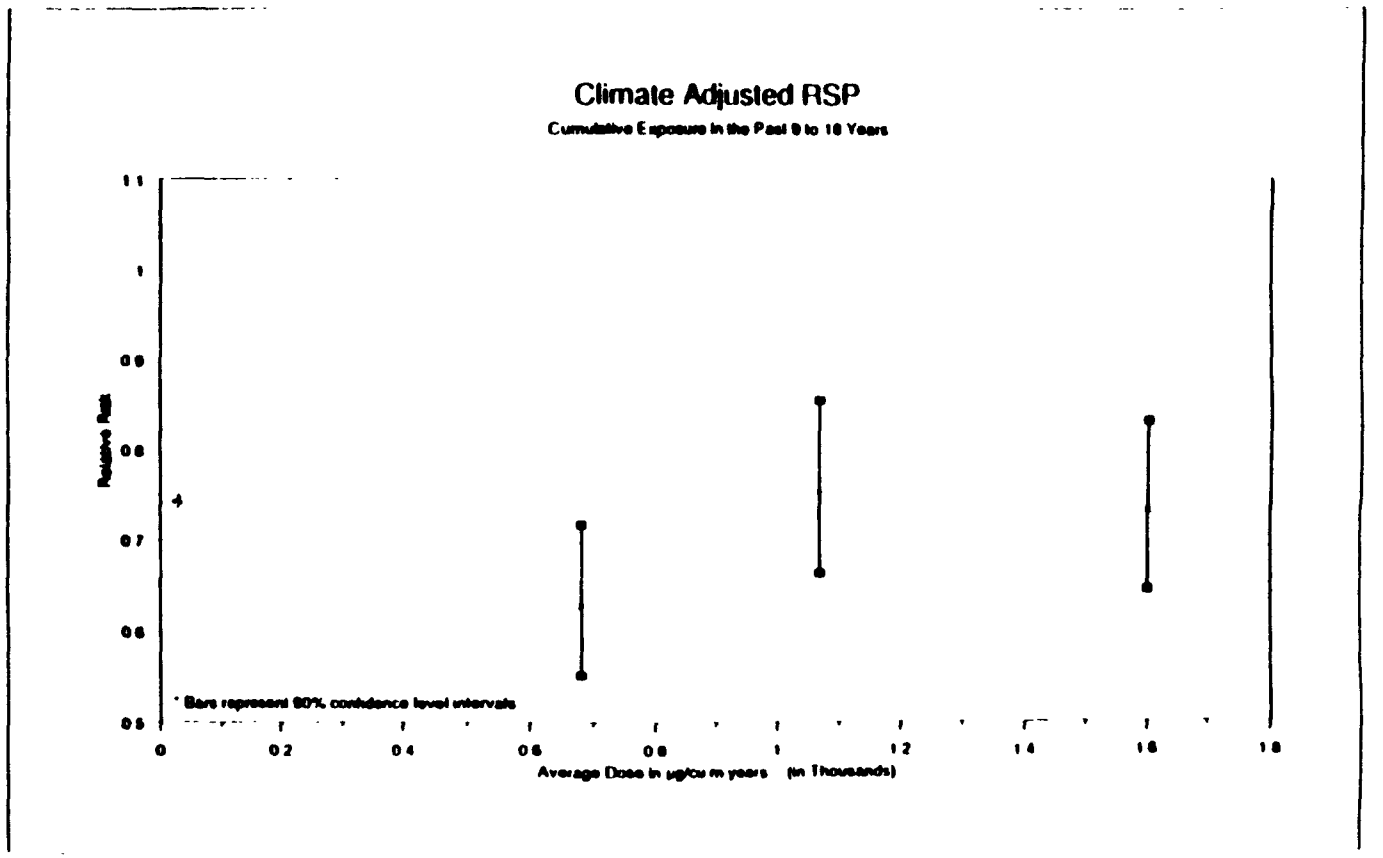
# Figure A4.3


Climate Adjusted RSP
Cumulative Exposure in the Past 4 to 8 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

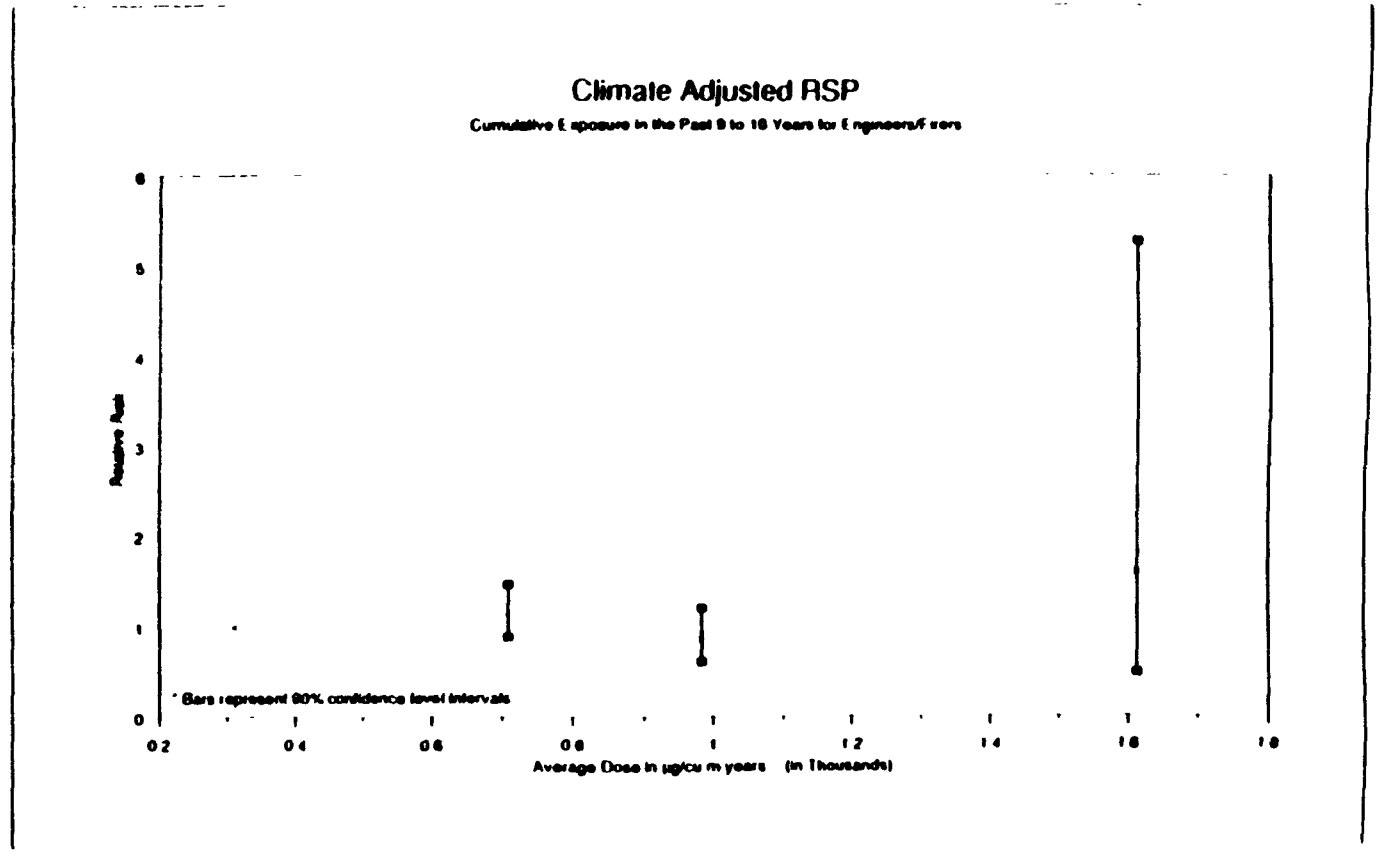
# Figure A4.4


Climate Adjusted RSP
Cumulative Exposure in the Past 4 to 8 Years for Shop Workers
Relative Risk
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
Average Dose in µg/cu m years (in Thousands)

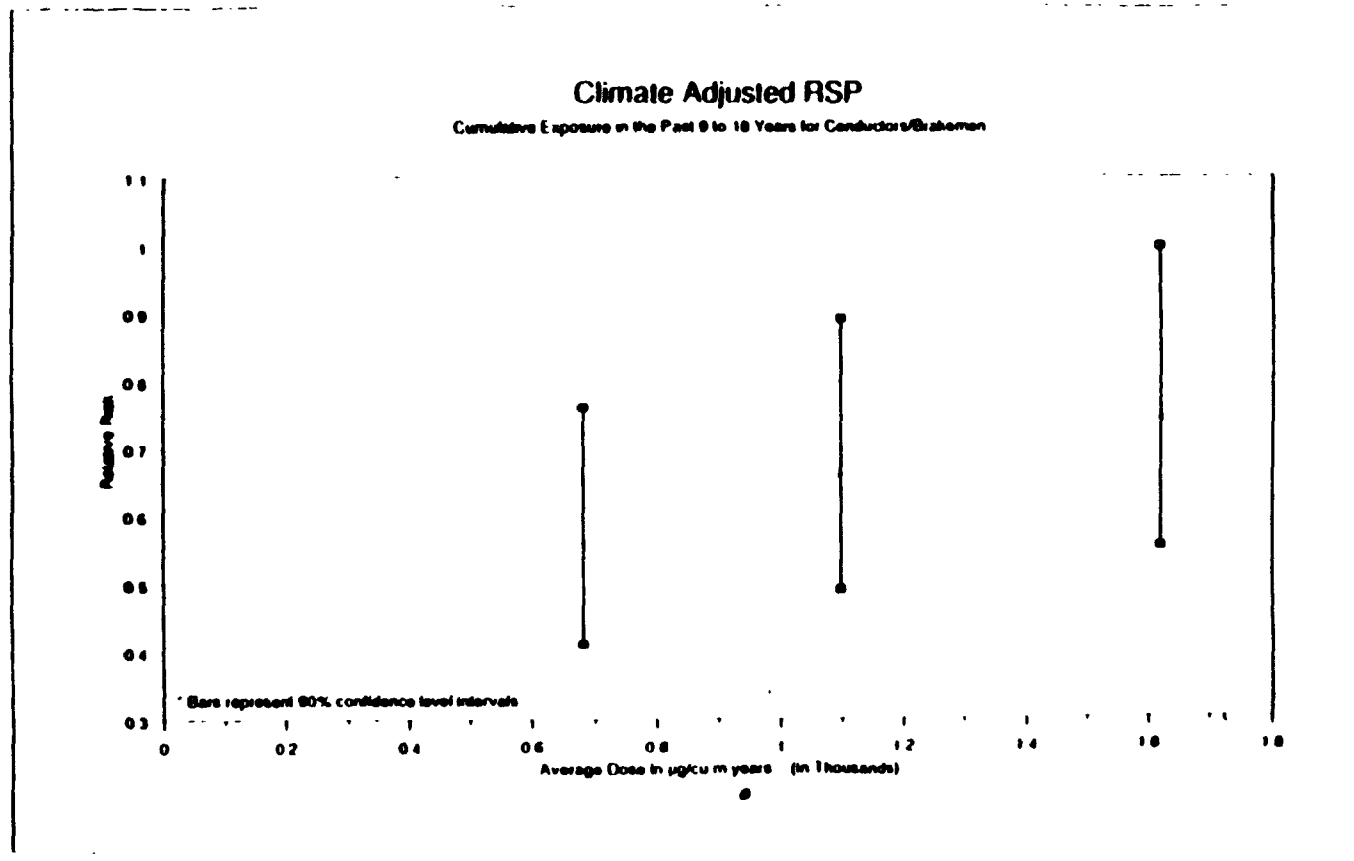
# Figure A4.5


Climate Adjusted RSP
Cumulative Exposure in the Past 9 to 18 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Dose in µg/cu m years (in Thousands)

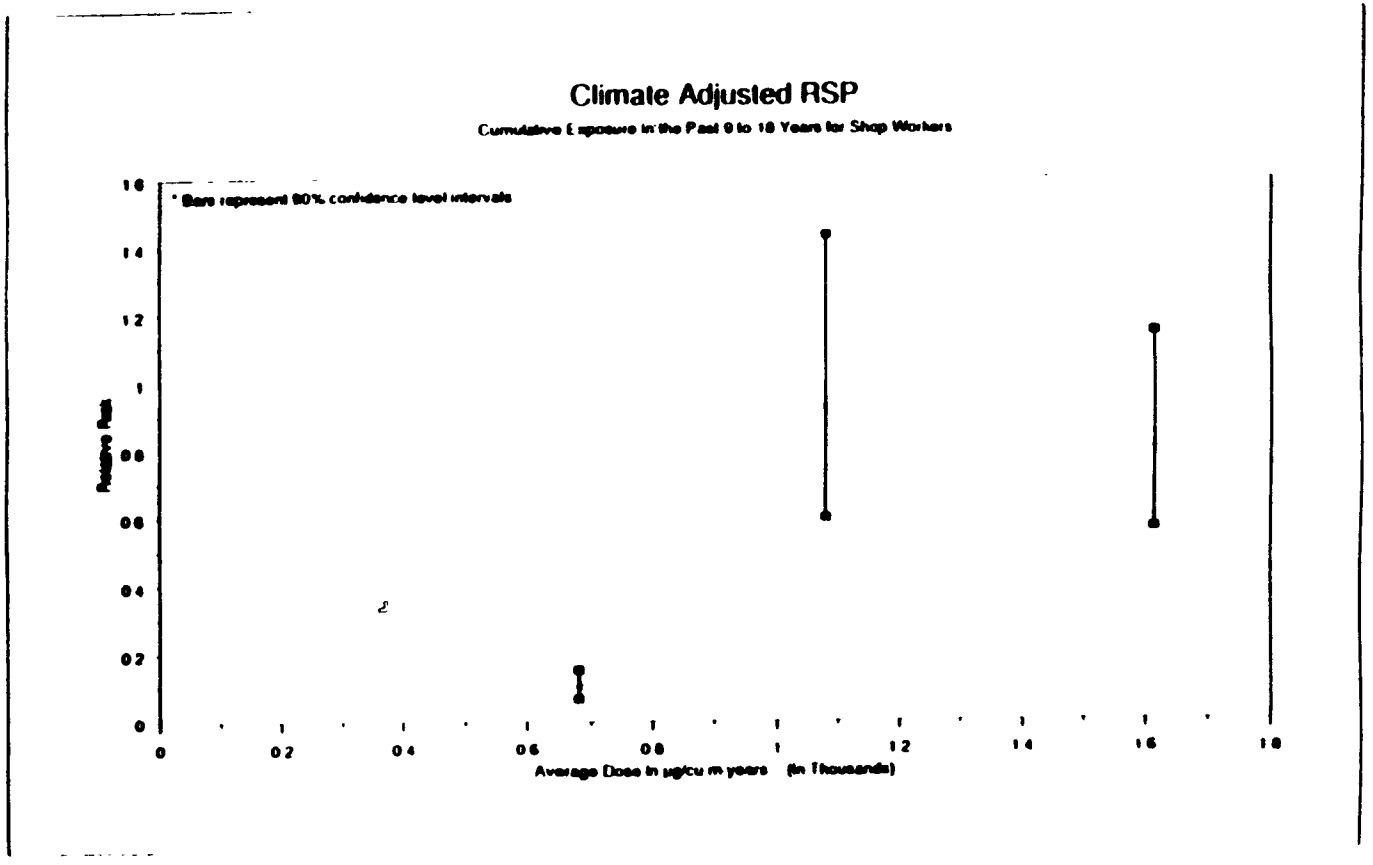
# Figure A4.6


Climate Adjusted RSP
Cumulative Exposure in the Past 9 to 18 Years for Engineers/Foreman
Relative Risk
0
1
2
3
4
5
6
* Bars represent 90% confidence level intervals
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Dose in µg/cu m years (in Thousands)

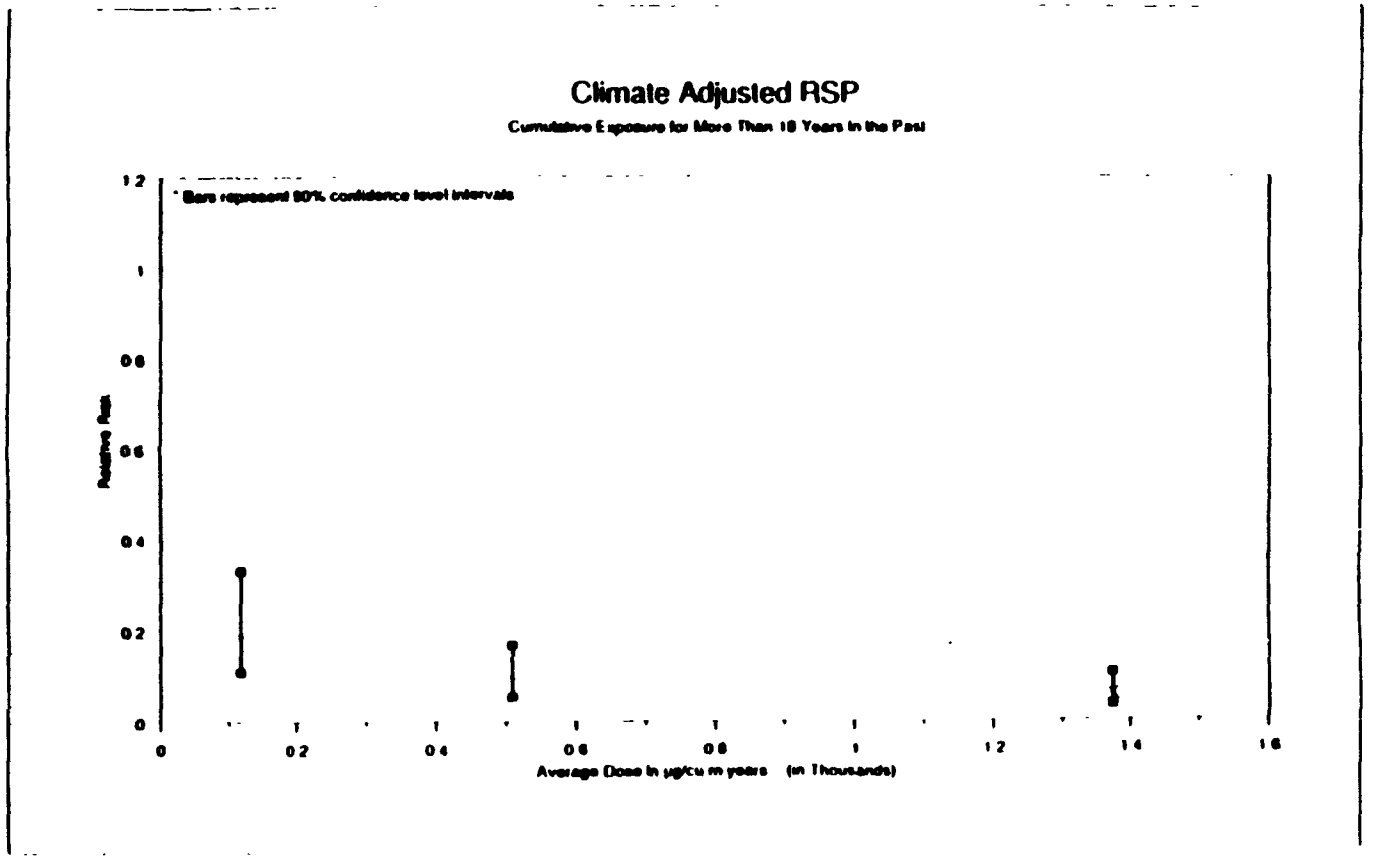
# Figure A4.7


Climate Adjusted RSP
Cumulative Exposure in the Past 9 to 18 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Dose in µg/cu m years (in Thousands)

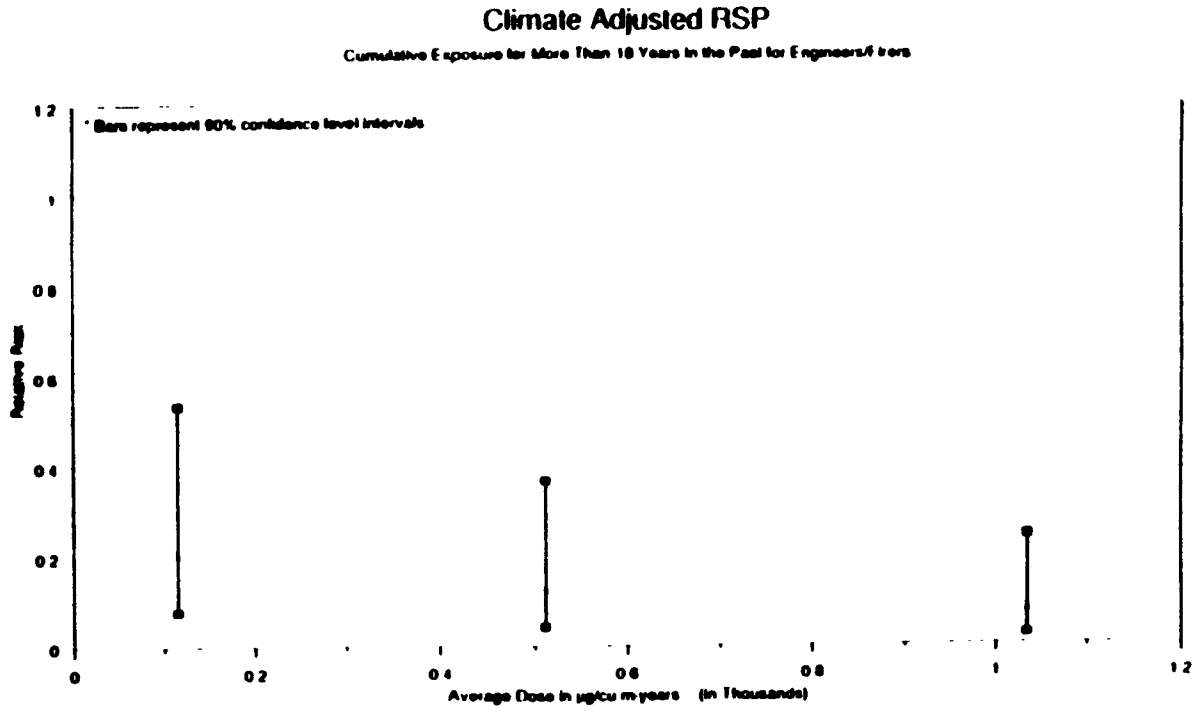
# Figure A4.8


Climate Adjusted RSP
Cumulative Exposure in the Past 9 to 18 Years for Shop Workers
* Bars represent 90% confidence level intervals
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Average Dose in µg/cu m years (in Thousands)

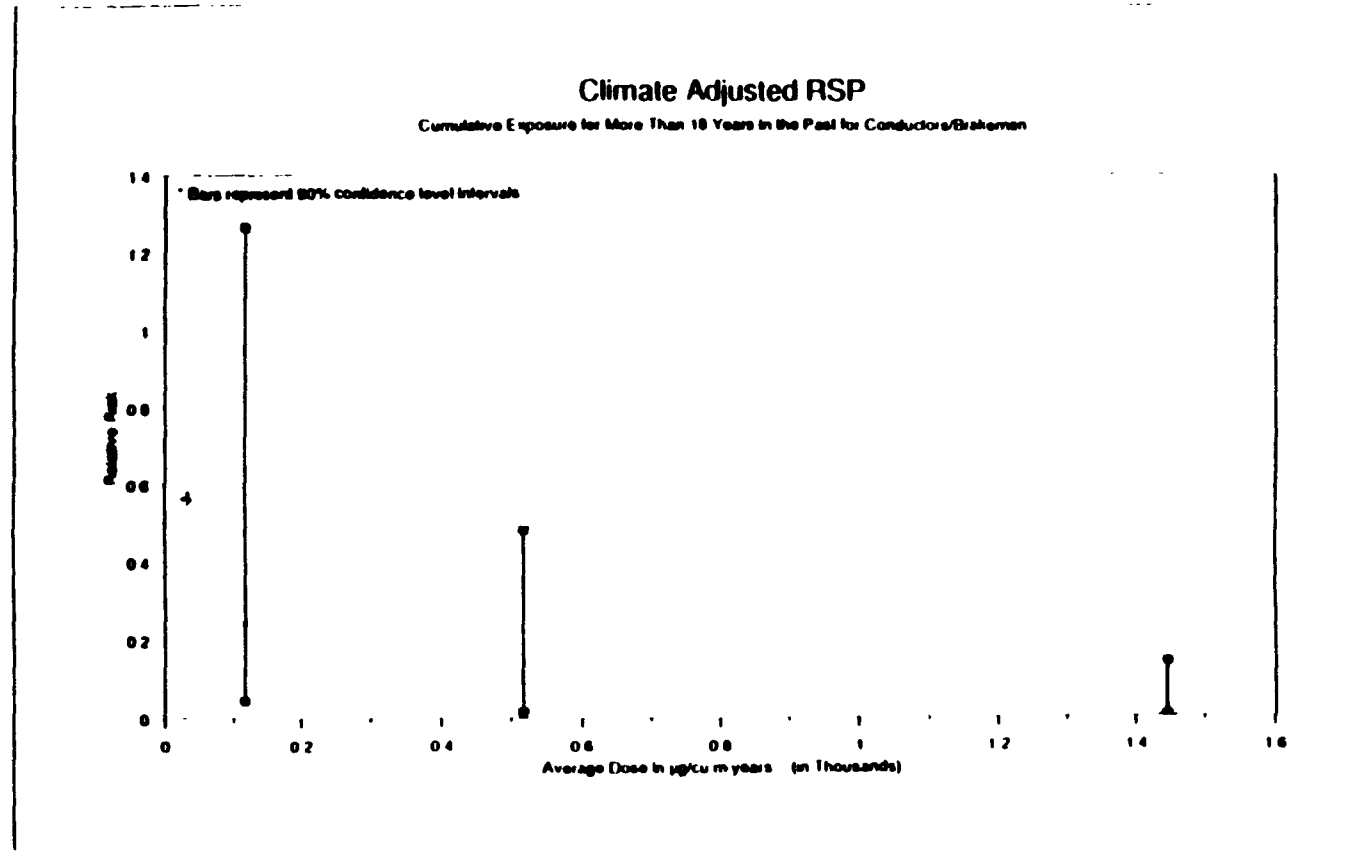
# Figure A4.9


Climate Adjusted RSP
Cumulative Exposure for More Than 18 Years in the Past
* Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Average Dose in µg/cu m years (in Thousands)

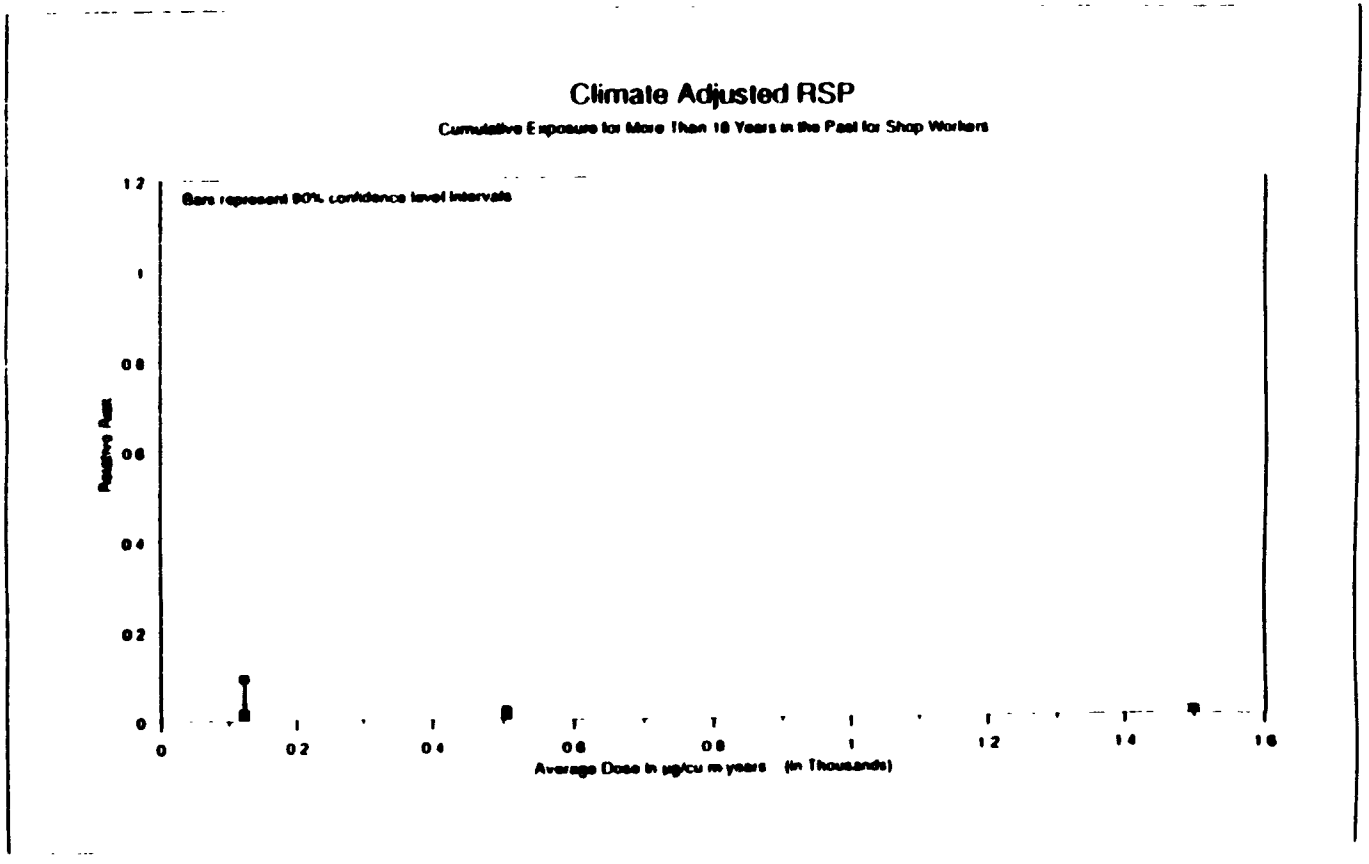
# Figure A4.10


Climate Adjusted RSP
Cumulative Exposure for More Than 18 Years in the Past for Engineers/Fitters
* Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m-years (in Thousands)

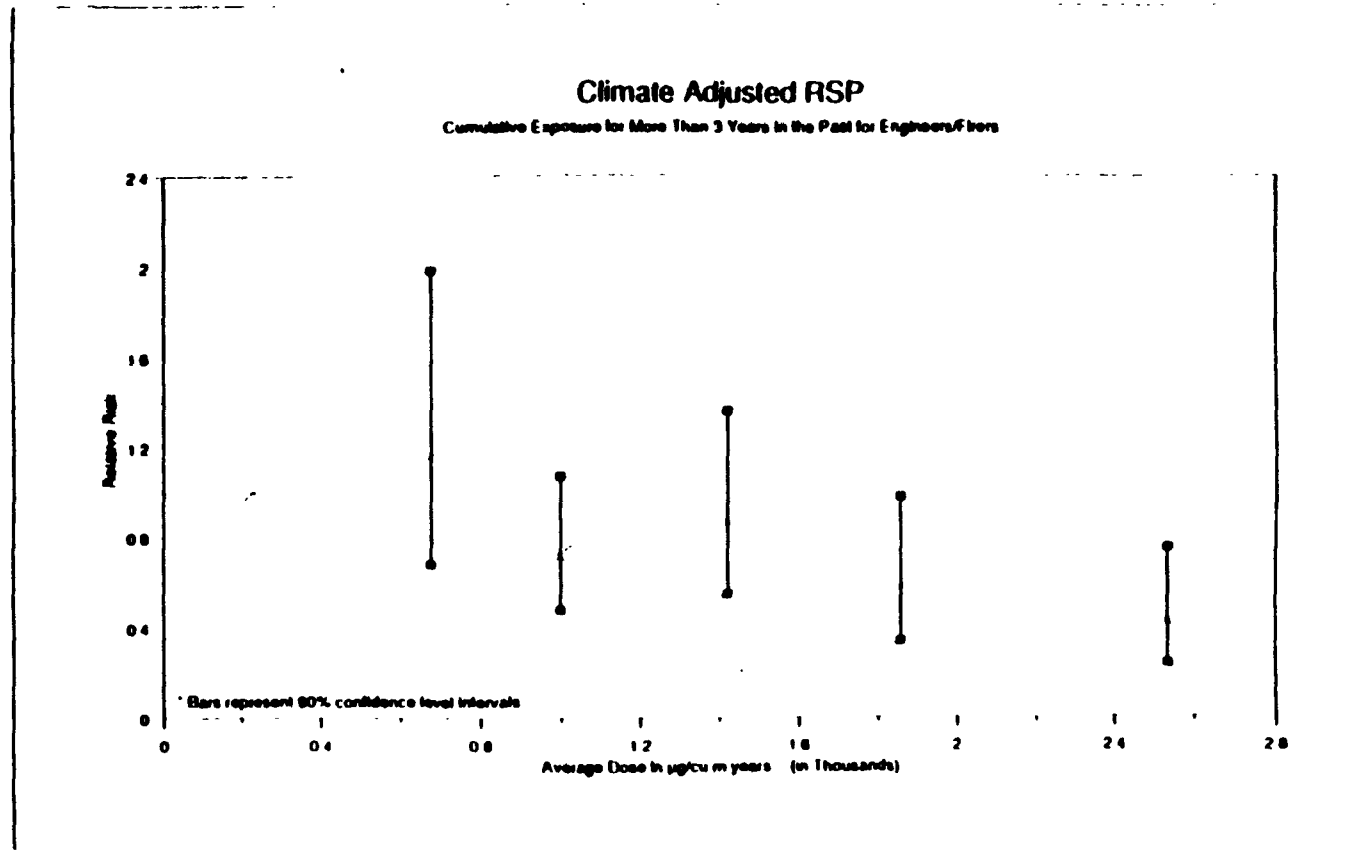
# Figure A4.11


Climate Adjusted RSP
Cumulative Exposure for More Than 10 Years in the Past for Conductors/Brakemen
* Bars represent 90% confidence level intervals
Relative Risk
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Average Dose in µg/cu m years (in Thousands)

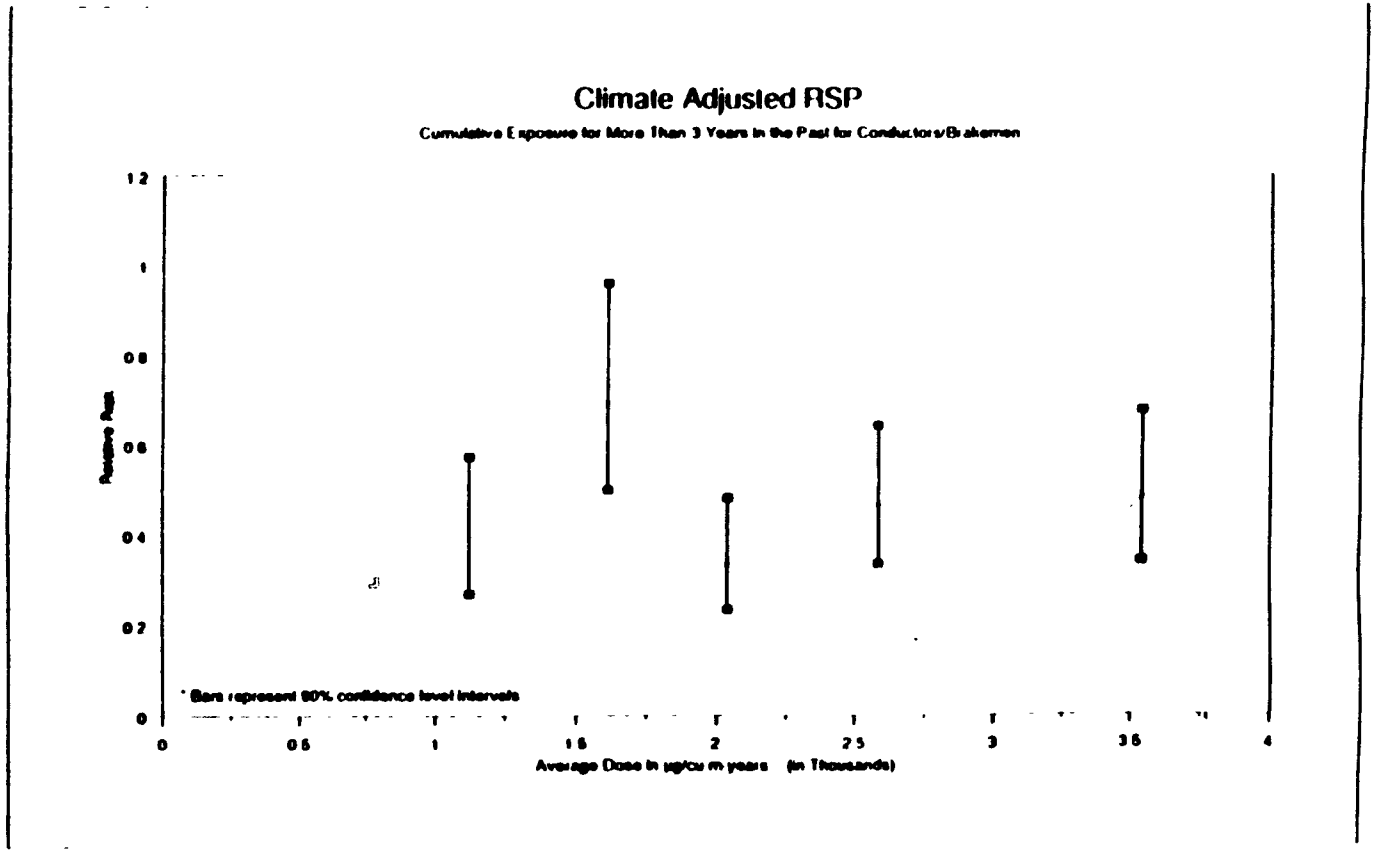
# Figure A4.12


Climate Adjusted RSP
Cumulative Exposure for More Than 10 Years in the Past for Shop Workers
Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
Average Dose in µg/cu m years (in Thousands)

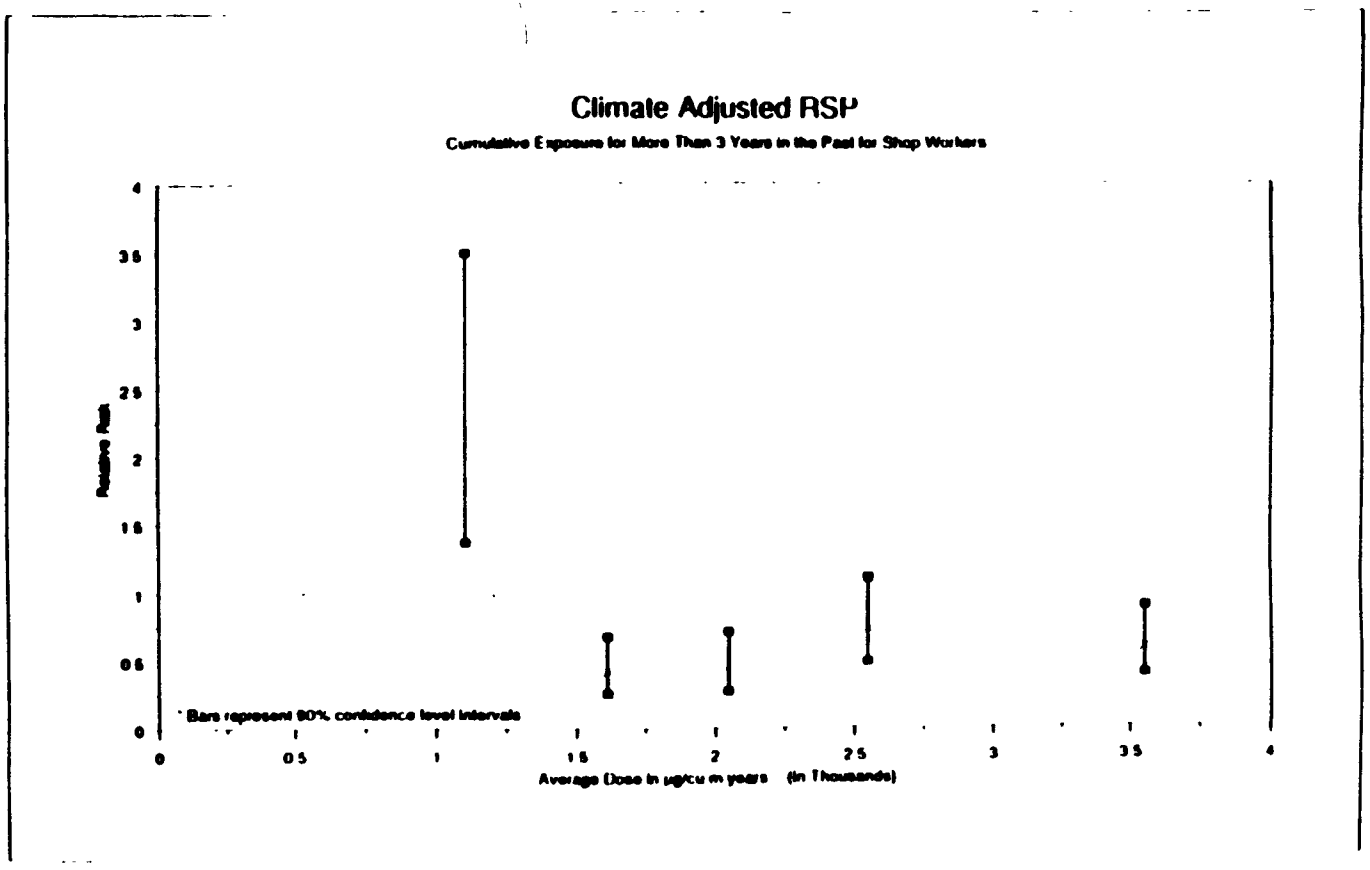
# Figure A4.13


Climate Adjusted RSP
Cumulative Exposure for More Than 3 Years in the Past for Engineers/Fibers
Relative Risk
2.4
2
1.6
1.2
0.8
0.4
0
* Bars represent 90% confidence level intervals
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Average Dose in µg/cu m years (in Thousands)

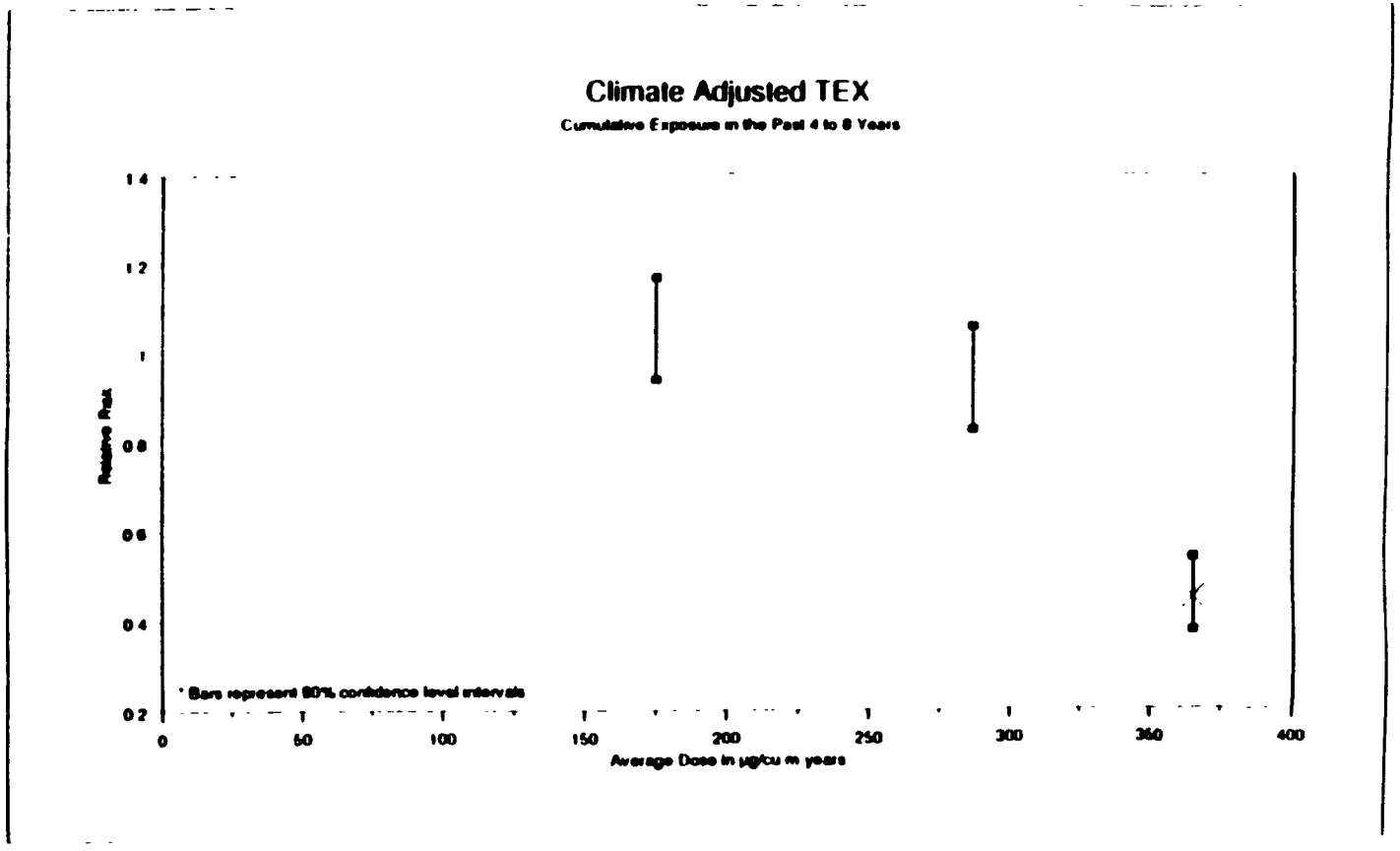
# Figure A4.14


Climate Adjusted RSP
Cumulative Exposure for More Than 3 Years in the Past for Conductors/Brakemen
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.5
1
1.5
2
2.5
3
3.5
4
Average Dose in µg/cu m years (in Thousands)

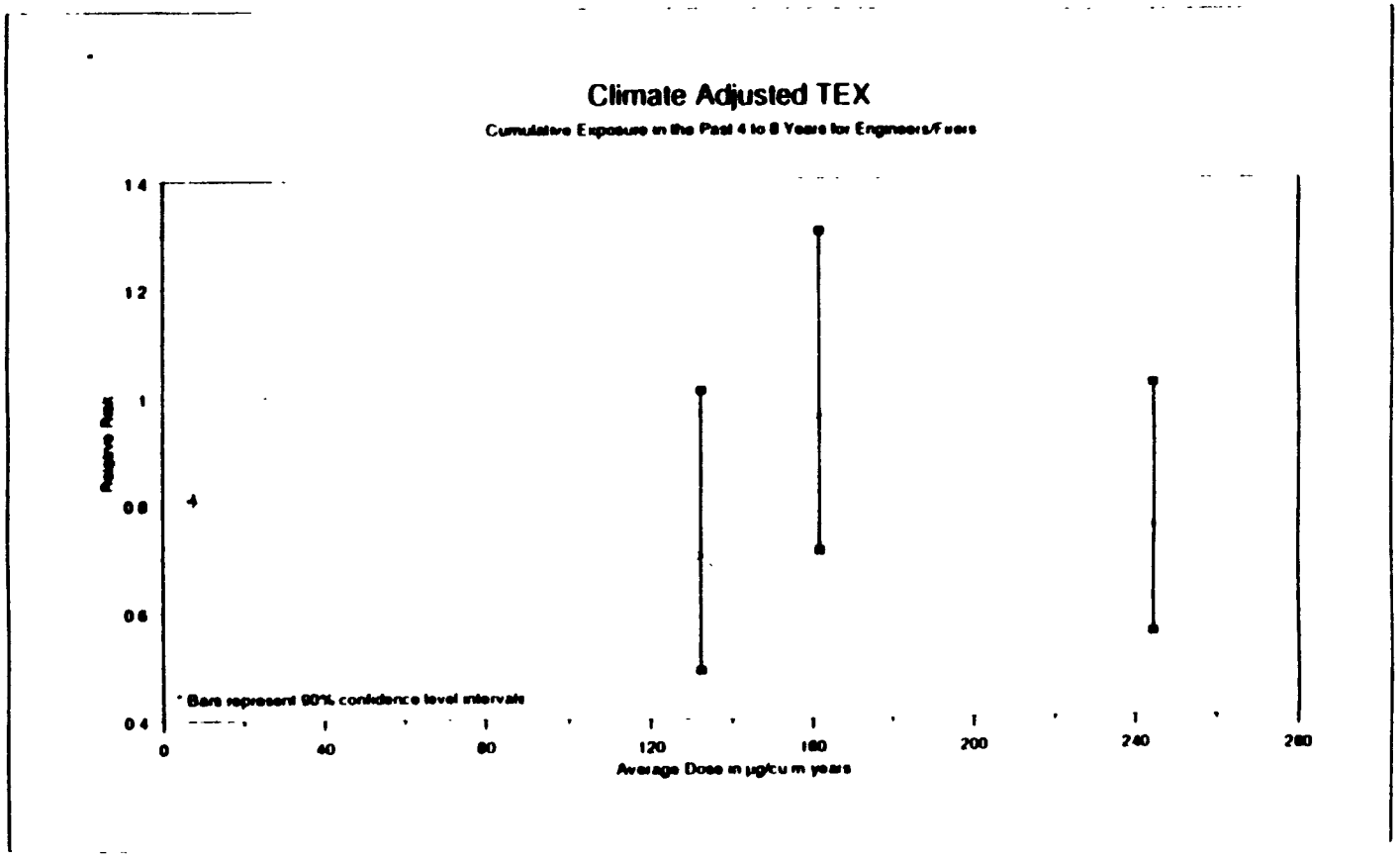
# Figure A4.15


Climate Adjusted RSP
Cumulative Exposure for More Than 3 Years in the Past for Shop Workers
Relative Risk
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Average Dose in µg/cu m years (in Thousands)
* Bars represent 90% confidence level intervals

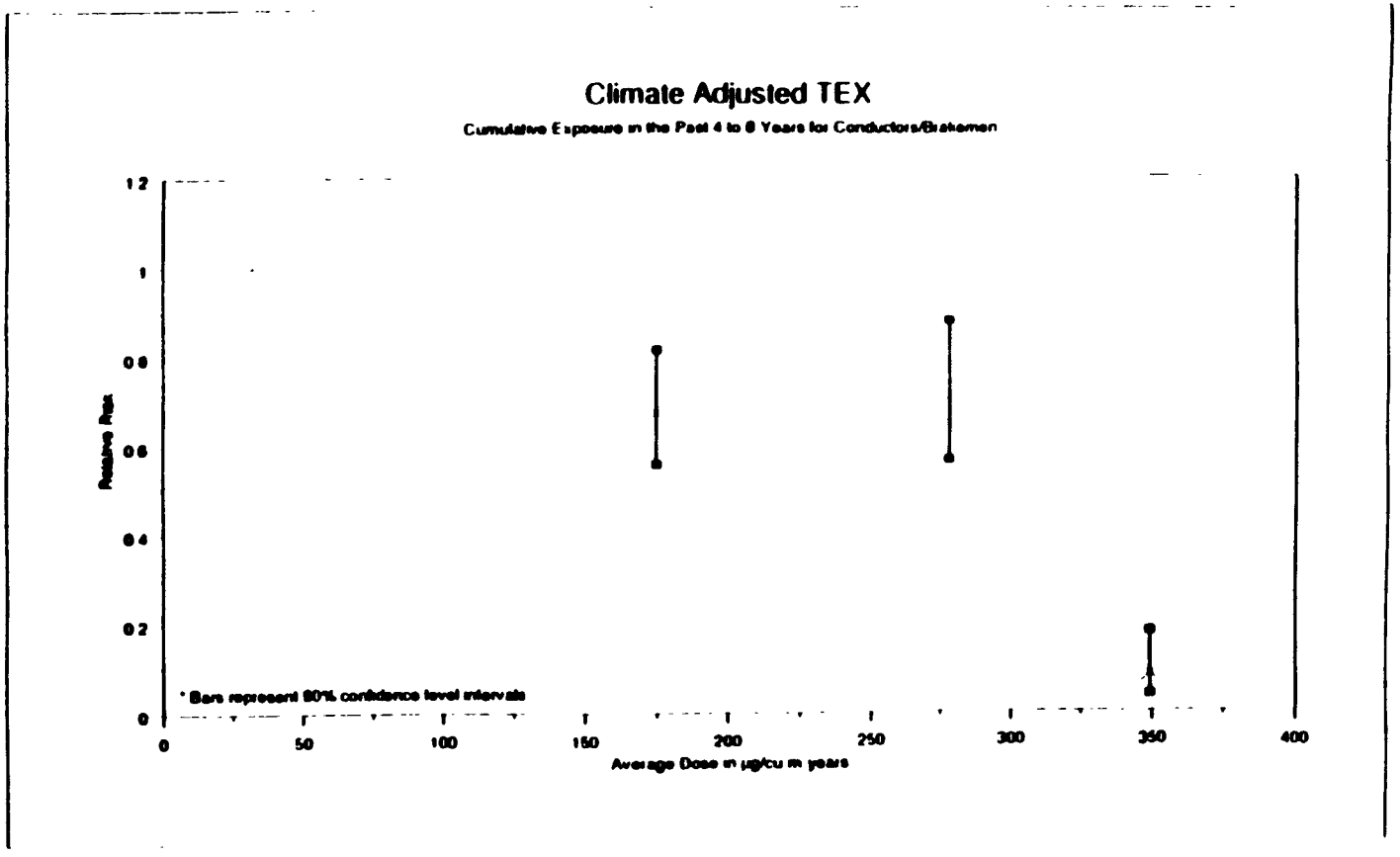
# Figure A5.1


Climate Adjusted TEX
Cumulative Exposure in the Past 4 to 8 Years
Relative Risk
1.4
1.2
1
0.8
0.6
0.4
0.2
* Bars represent 90% confidence level intervals
0
50
100
150
200
250
300
350
400
Average Dose in µg/cu m years

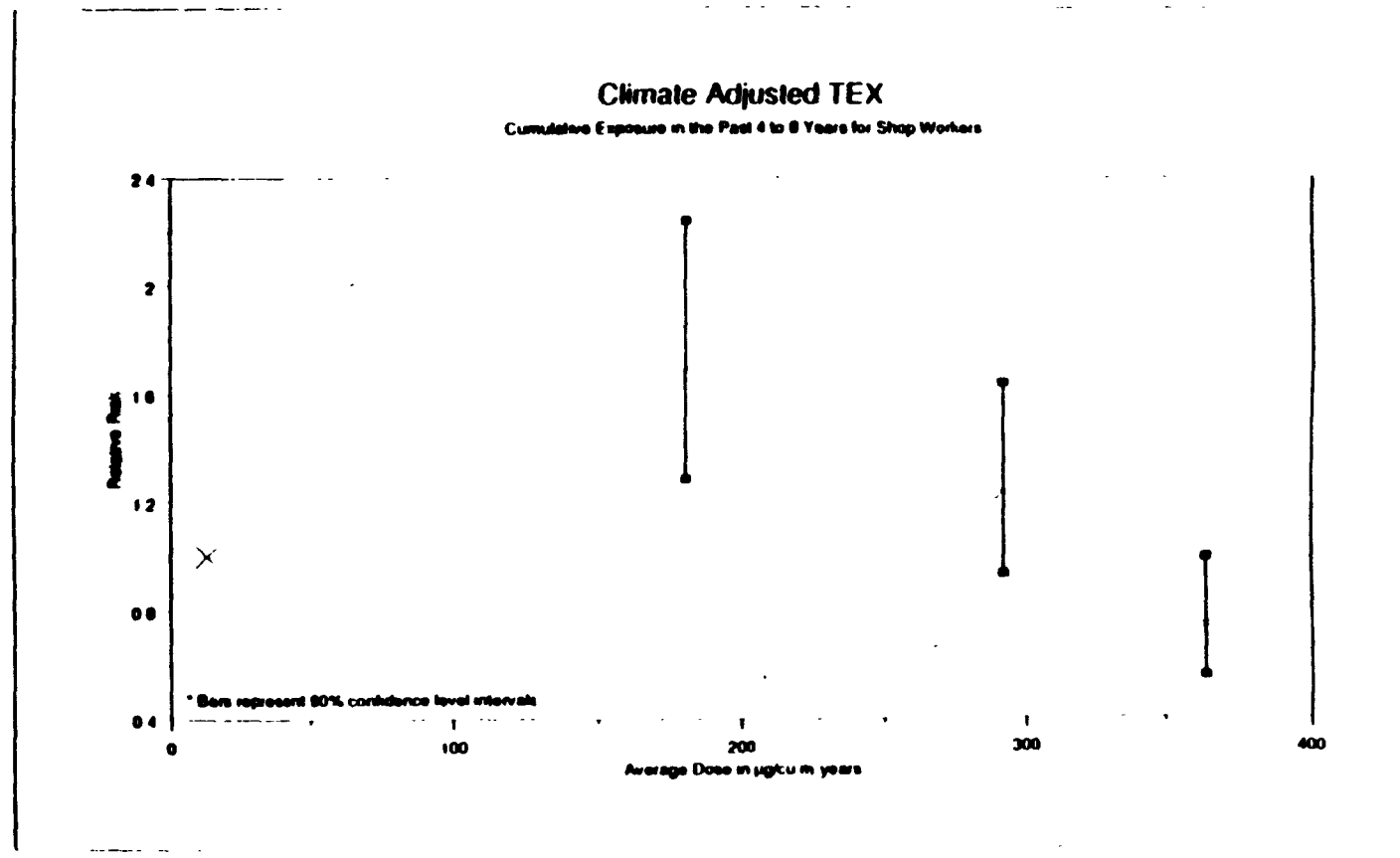
# Figure A5.2


Climate Adjusted TEX
Cumulative Exposure in the Past 4 to 8 Years for Engineers/Fusers
Response Rate
1.4
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
40
80
120
160
200
240
280
Average Dose in µg/cu m years

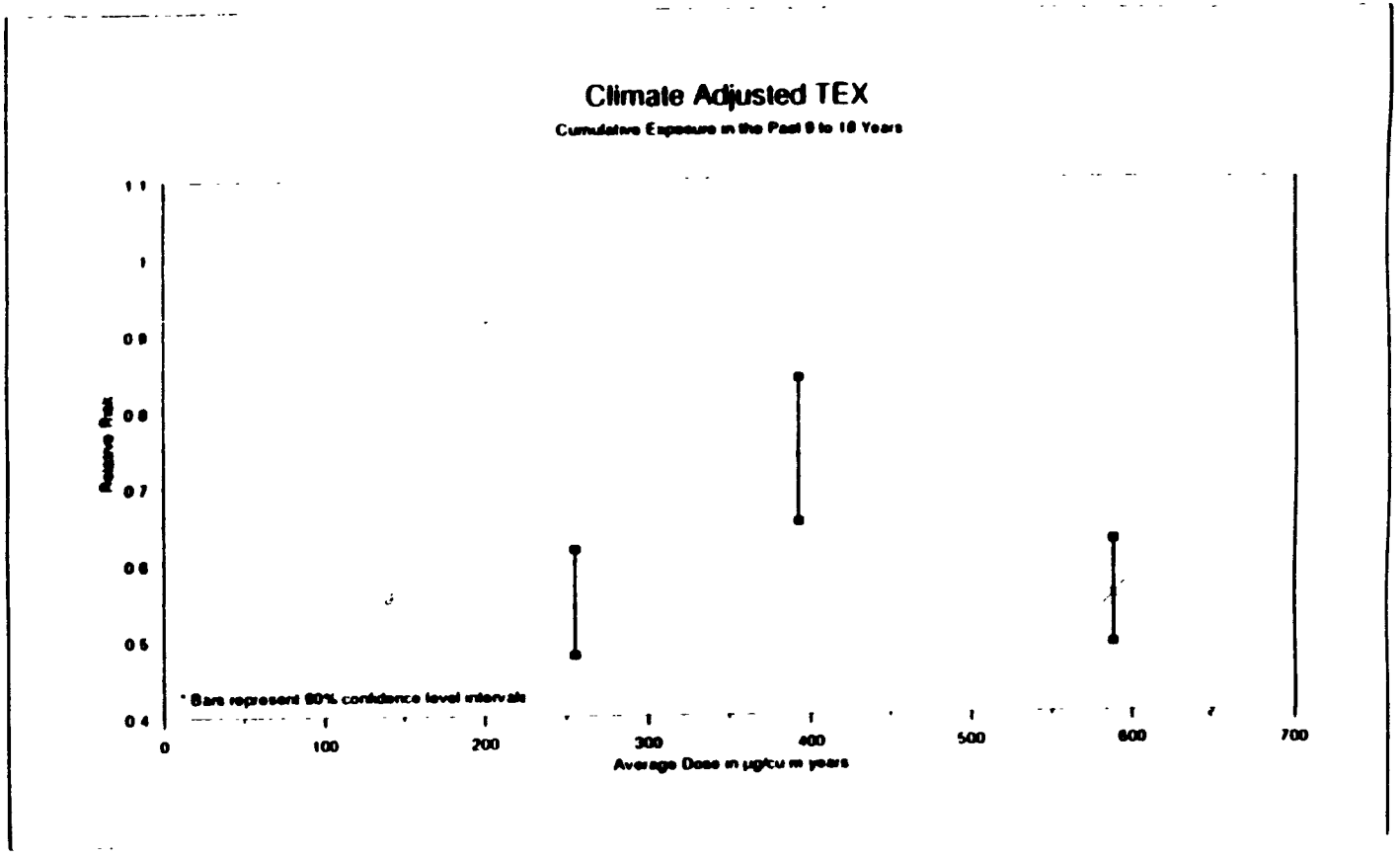
# Figure A5.3


Climate Adjusted TEX
Cumulative Exposure in the Past 4 to 8 Years for Conductors/Brakemen
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
50
100
150
200
250
300
350
400
Average Dose in µg/cu m years

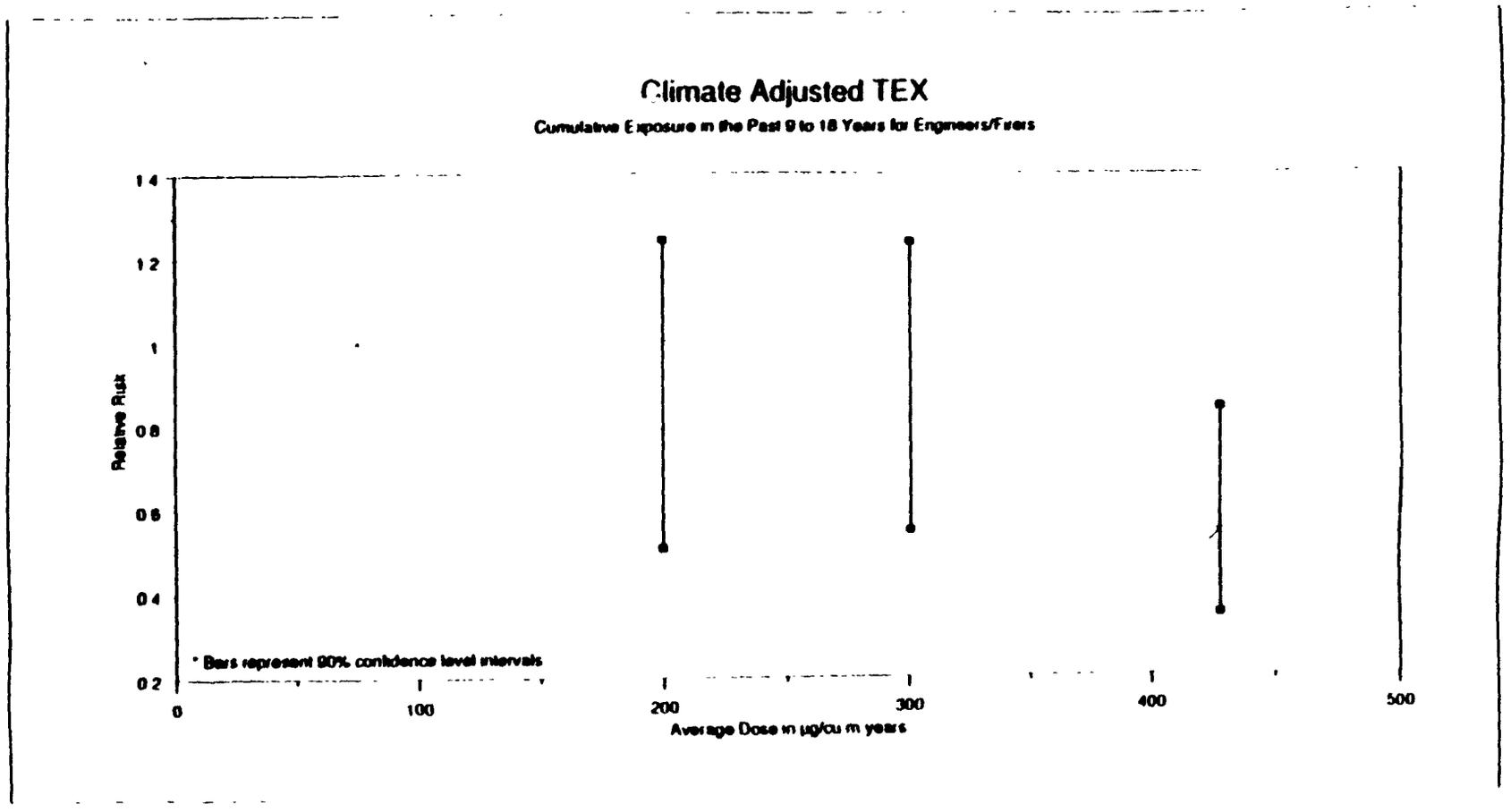
# Figure A5.4


Climate Adjusted TEX
Cumulative Exposure in the Past 4 to 8 Years for Shop Workers
Relative Risk
2.4
2
1.6
1.2
0.8
0.4
0
100
200
300
400
Average Dose in µg/cu m years
* Bars represent 90% confidence level intervals

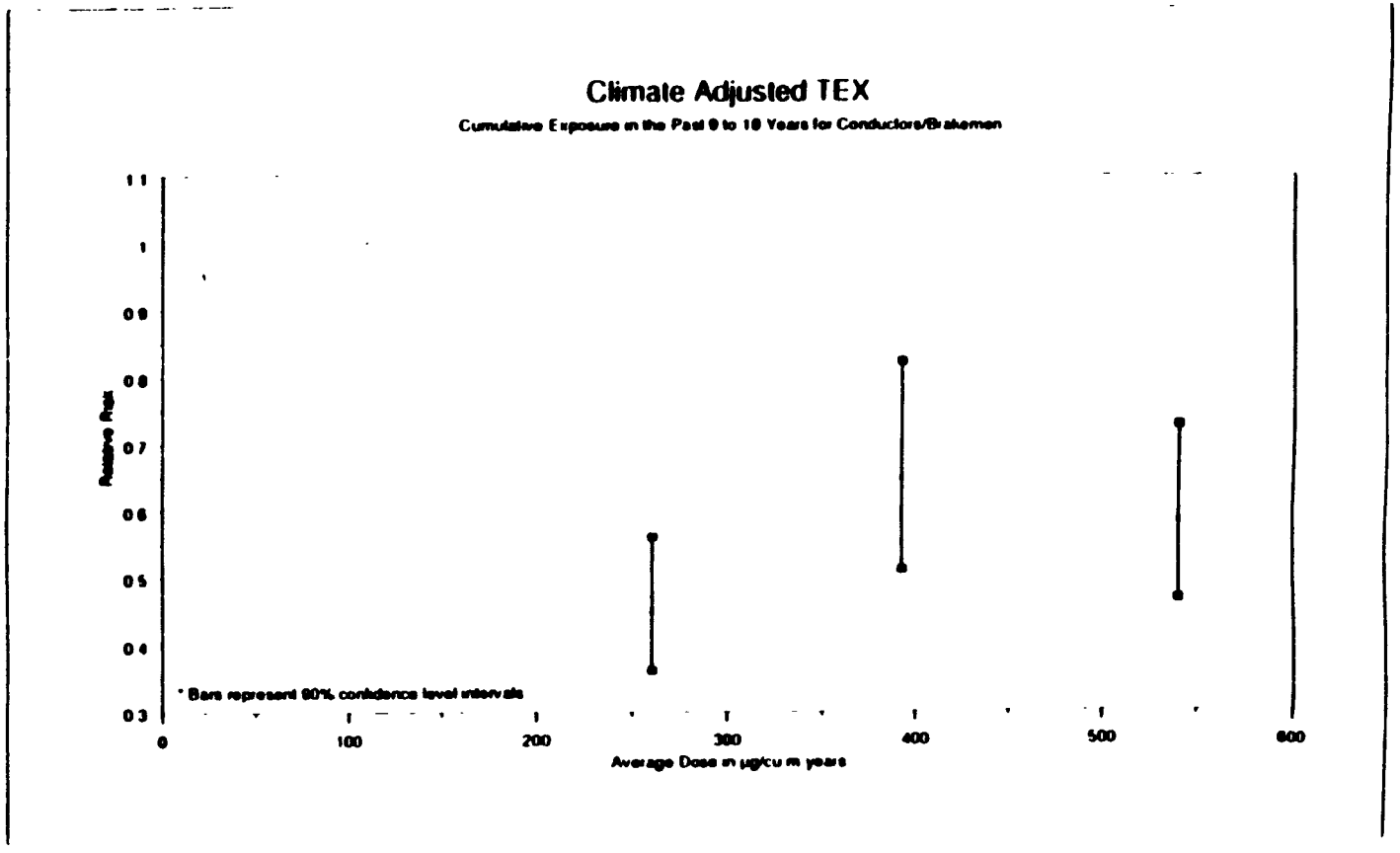
# Figure A5.5


Climate Adjusted TEX
Cumulative Exposure in the Past 9 to 18 Years
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
Average Dose in µg/cu m years

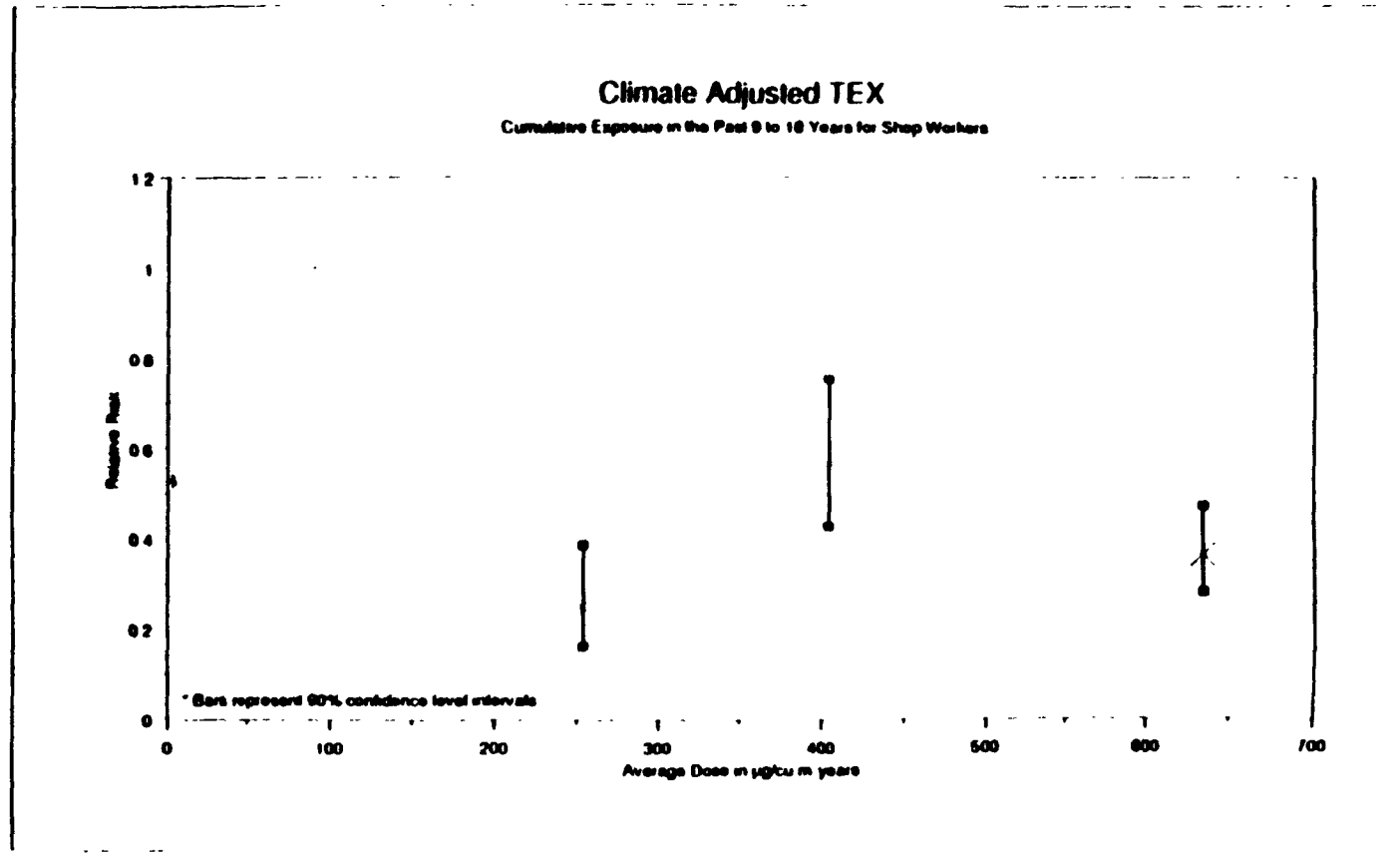
# Figure A5.6


Climate Adjusted TEX
Cumulative Exposure in the Past 9 to 18 Years for Engineers/Firers
Relative Risk
1.4
1.2
1
0.8
0.6
0.4
0.2
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
Average Dose in µg/cu m years

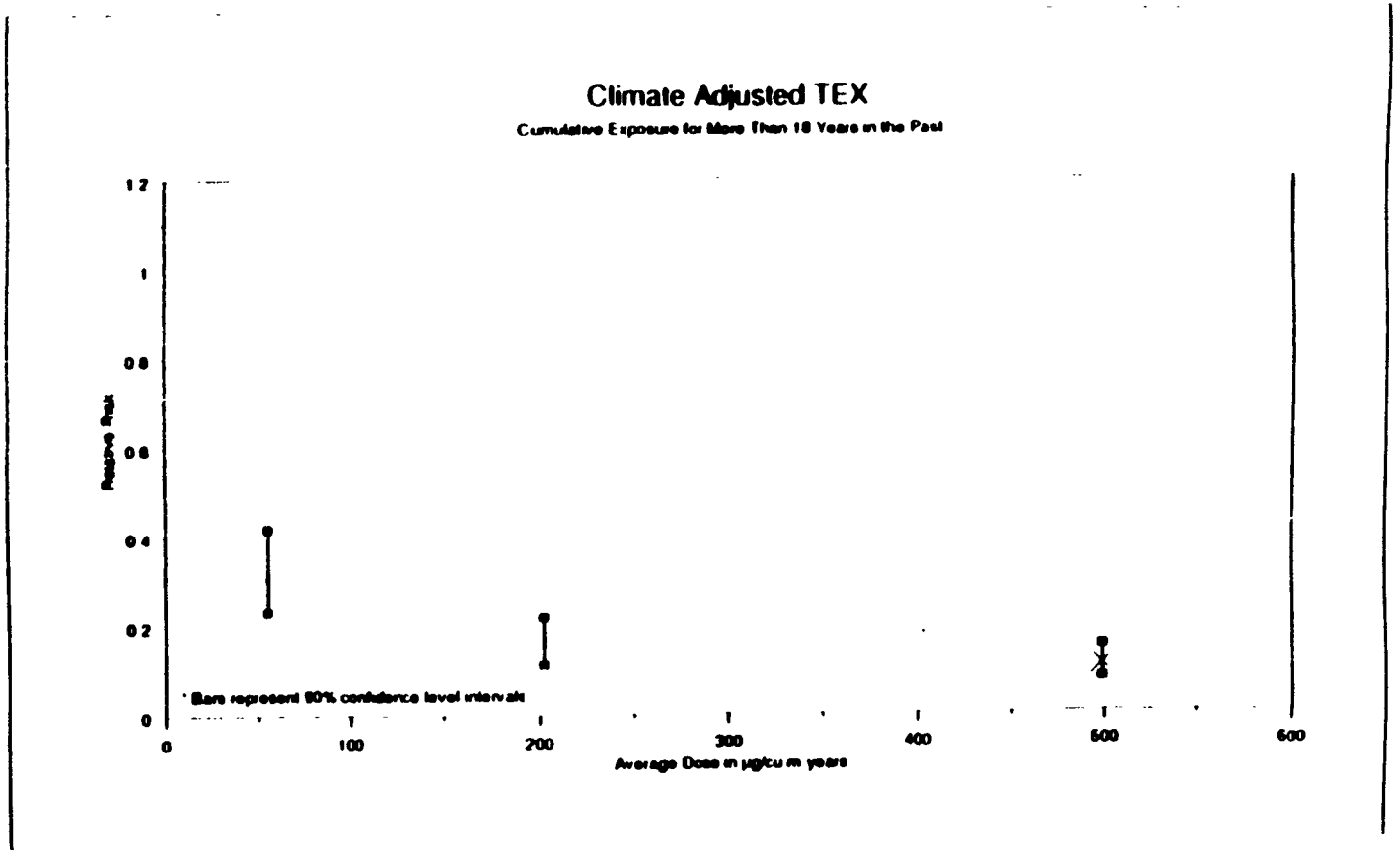
# Figure A5.7


Climate Adjusted TEX
Cumulative Exposure in the Past 9 to 18 Years for Conductors/Brakemen
Relative Risk
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
Average Dose in µg/cu m years

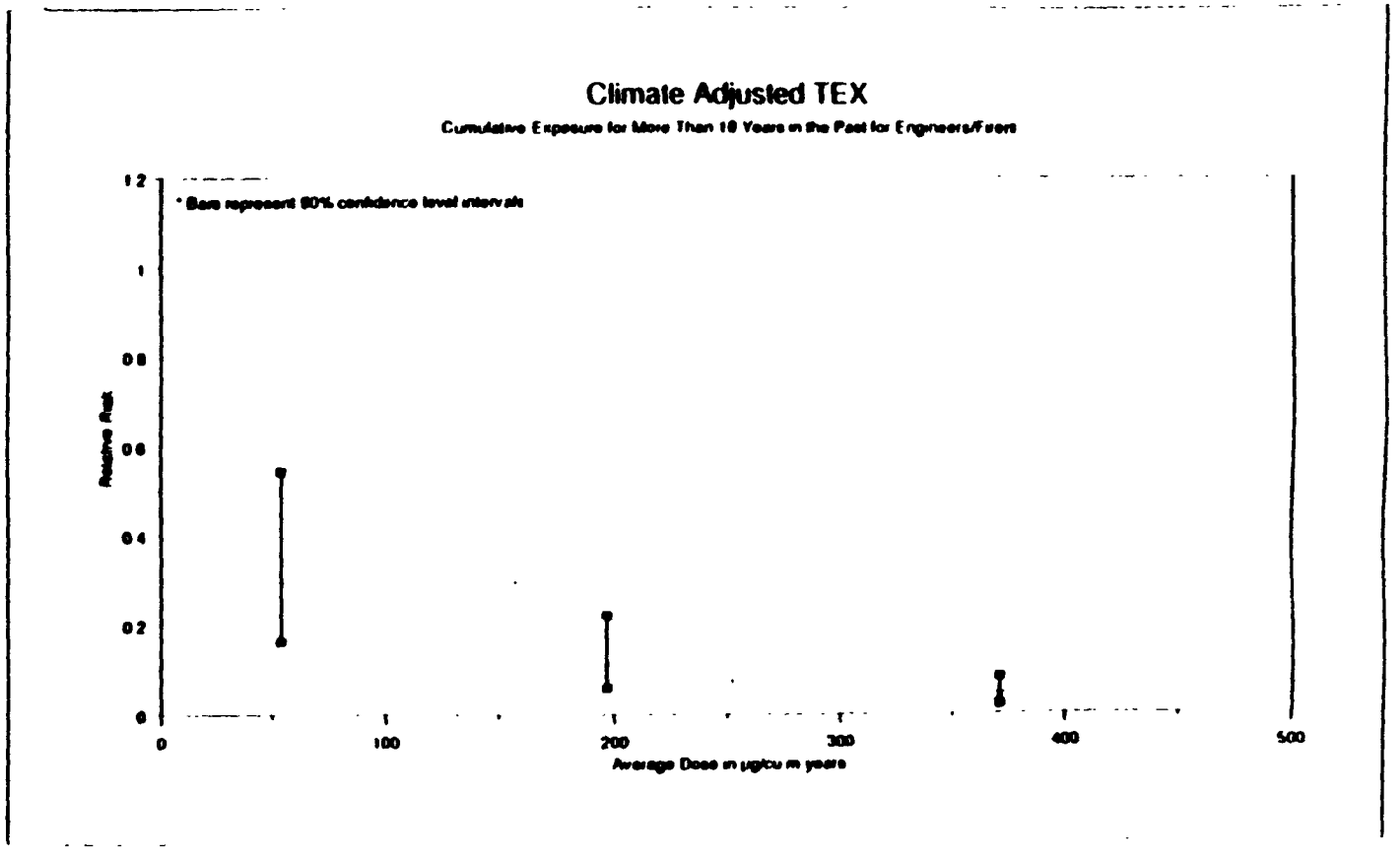
# Figure A5.8


Climate Adjusted TEX
Cumulative Exposure in the Past 9 to 18 Years for Shop Workers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
700
Average Dose in µg/cu m years

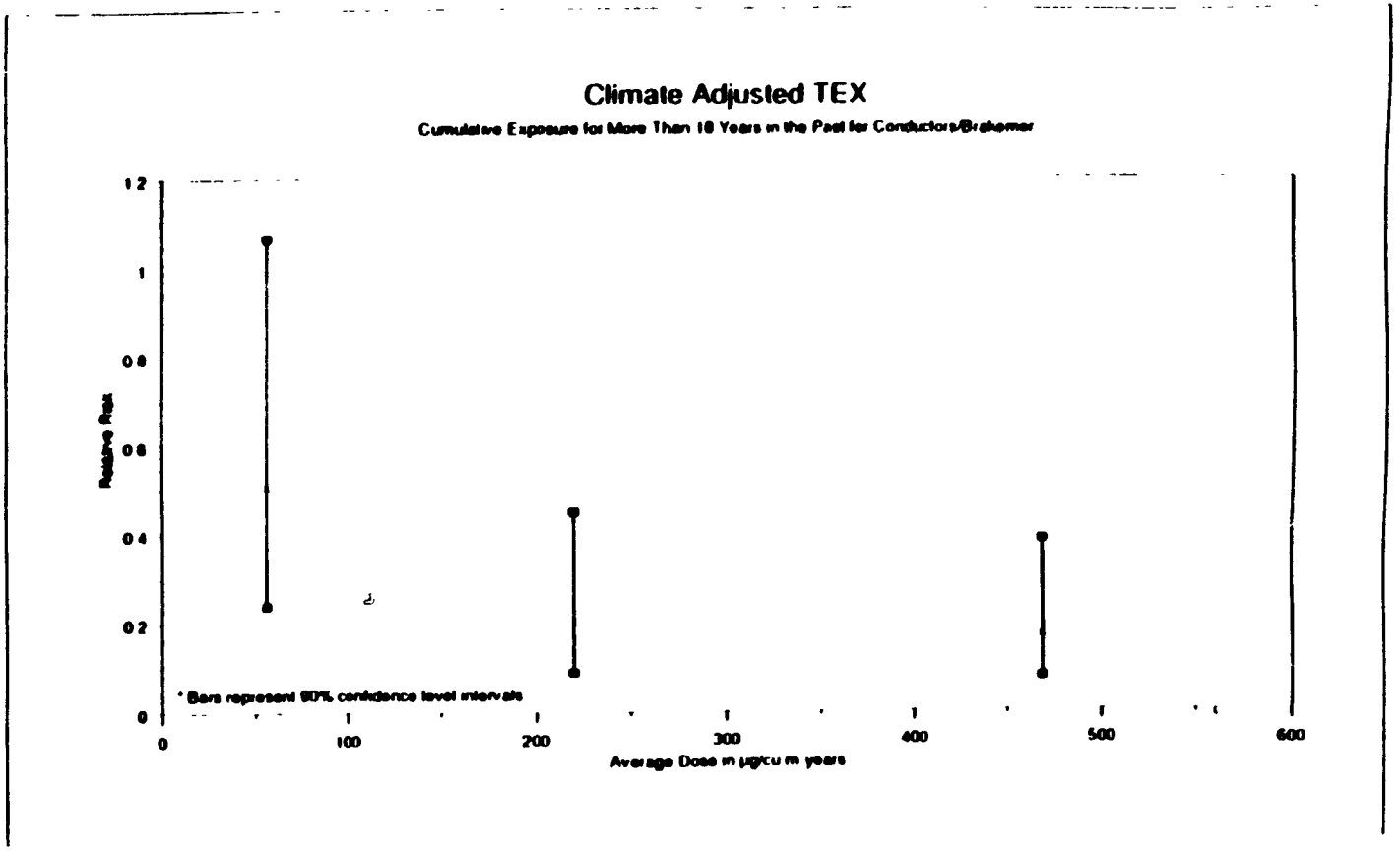
# Figure A5.9


Climate Adjusted TEX
Cumulative Exposure for More Than 18 Years in the Past
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
Average Dose in µg/cu m years

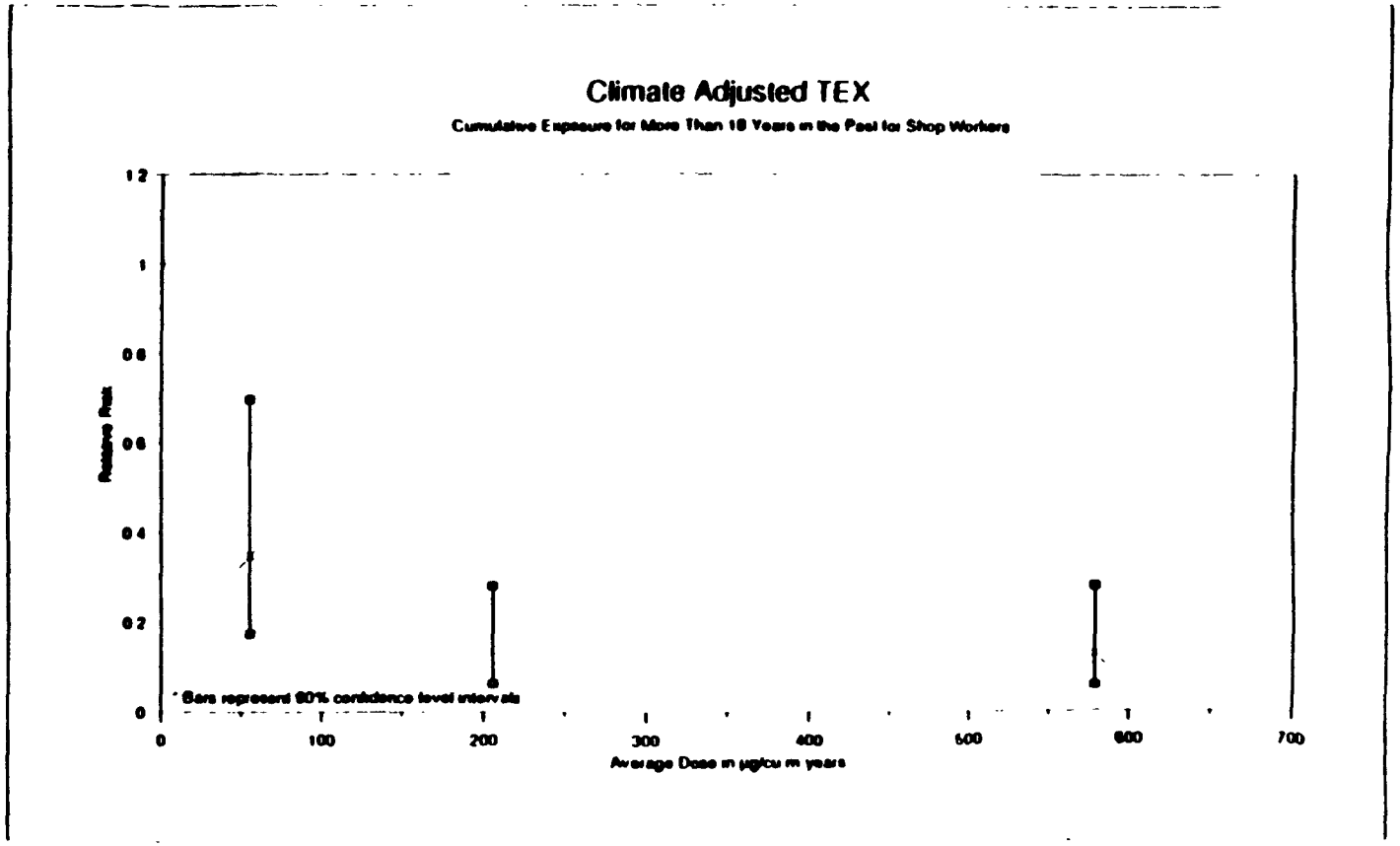
# Figure A5.10


Climate Adjusted TEX
Cumulative Exposure for More Than 10 Years in the Past for Engineers/Fixers
* Bars represent 90% confidence level intervals
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
Average Dose in µg/cu m years

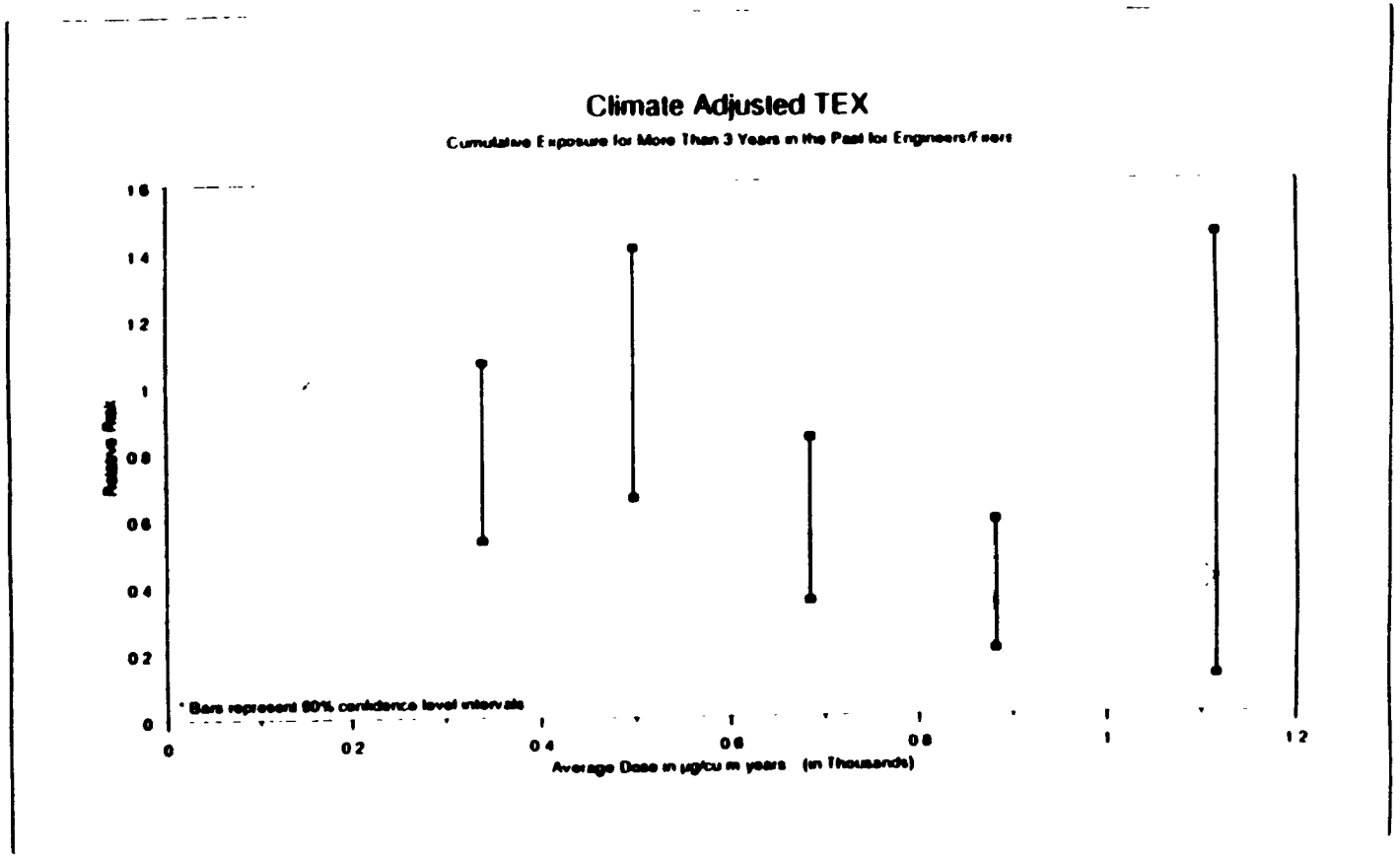
# Figure A5.11


Climate Adjusted TEX
Cumulative Exposure for More Than 10 Years in the Past for Conductors/Brakemen
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
500
600
Average Dose in µg/cu m years

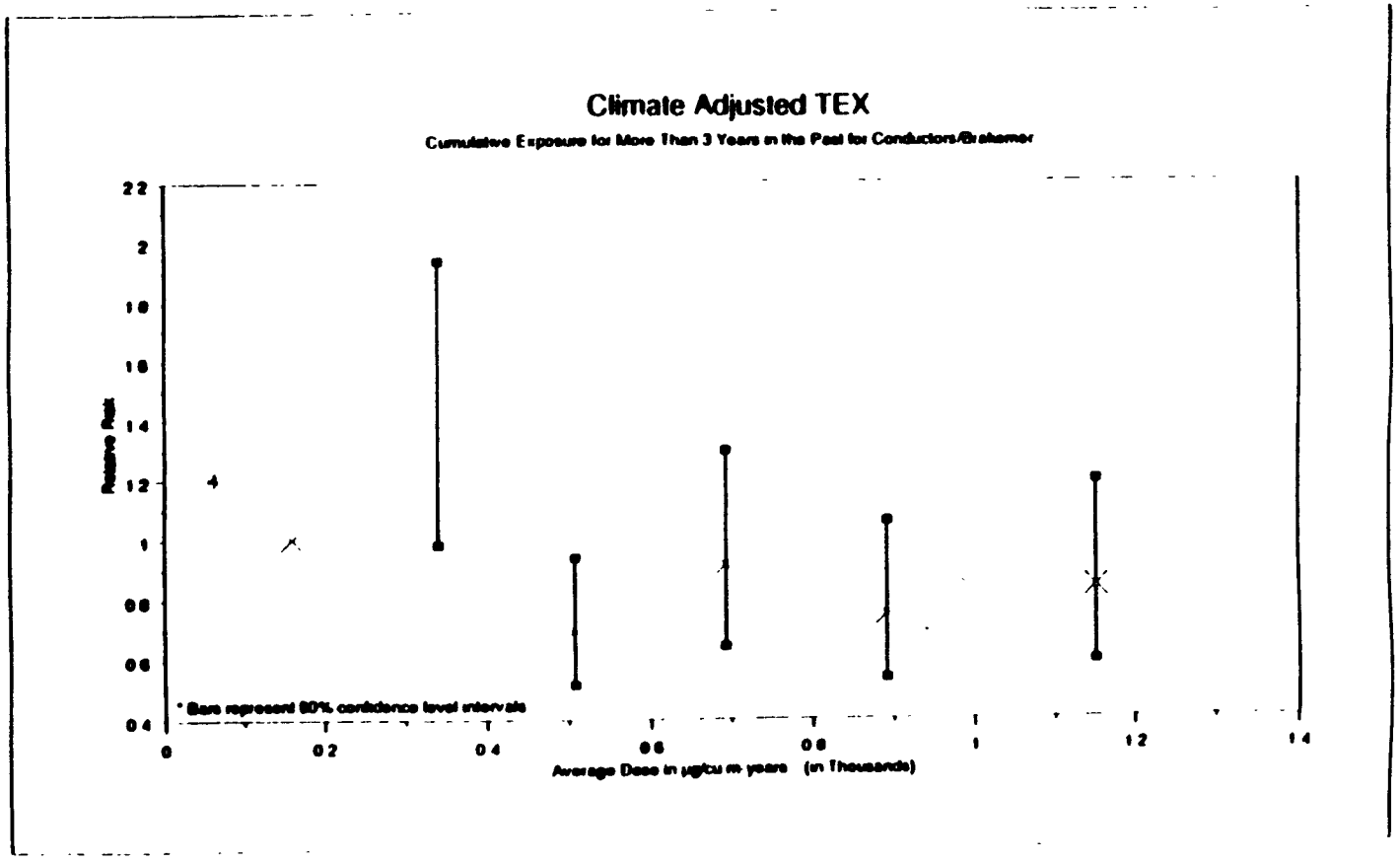
# Figure A5.12


Climate Adjusted TEX
Cumulative Exposure for More Than 18 Years in the Past for Shop Workers
Relative Risk
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
100
200
300
400
600
800
700
Average Dose in µg/cu m years

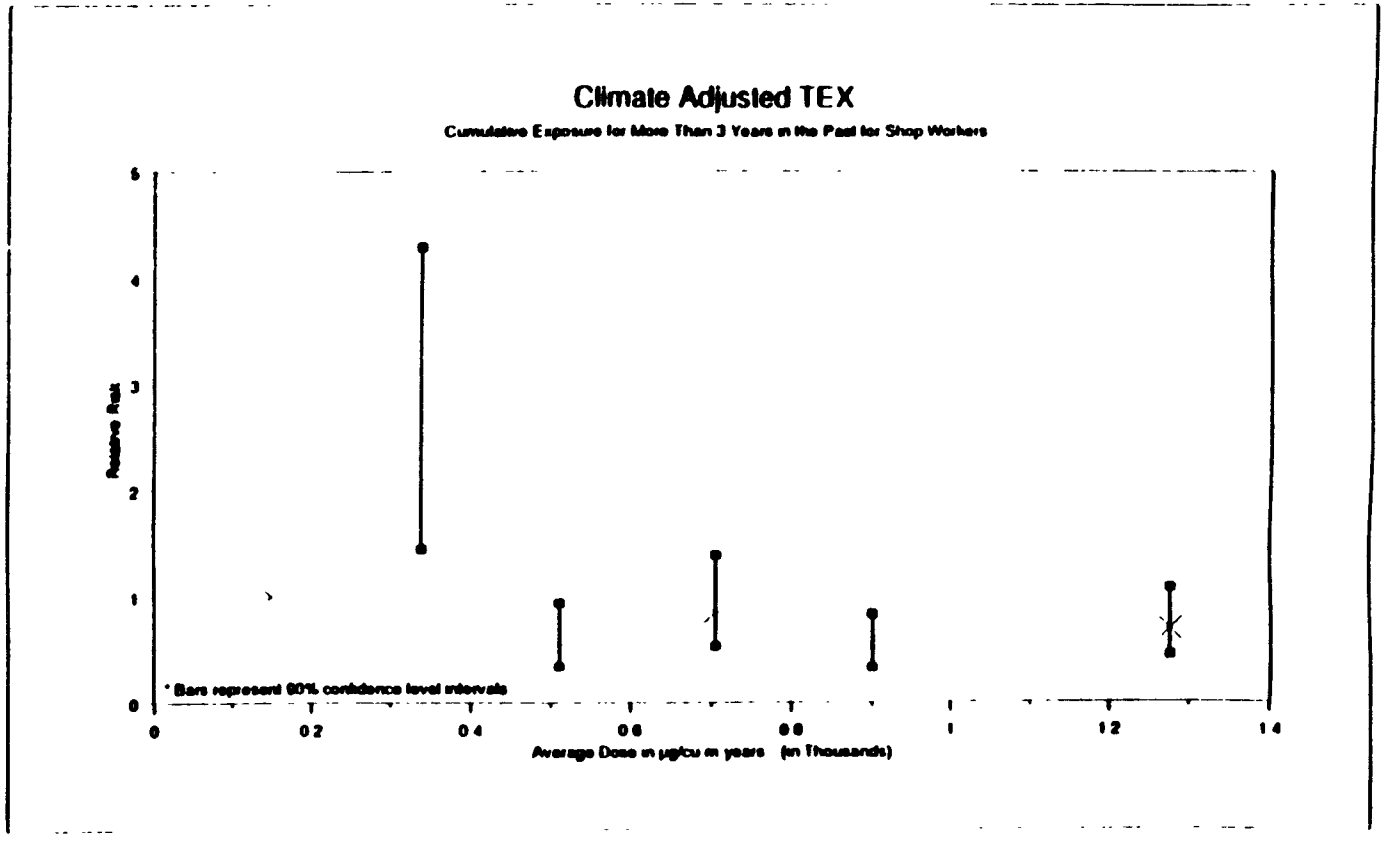
# Figure A5.13


Climate Adjusted TEX
Cumulative Exposure for More Than 3 Years in the Past for Engineers/Fitters
Relative Risk
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
Average Dose in µg/cu m years (in Thousands)

# Figure A5.14


Climate Adjusted TEX
Cumulative Exposure for More Than 3 Years in the Past for Conductors/Brakemen
Relative Risk
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average Dose in µg/cu m years (in Thousands)

# Figure A5.15


Climate Adjusted TEX
Cumulative Exposure for More Than 3 Years in the Past for Shop Workers
Relative Risk
0
1
2
3
4
5
* Bars represent 90% confidence level intervals
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Average Dose in µg/cu m years (in Thousands)

## ESTIMATES OF INCREASE IN RELATIVE RISKS IN LUNG CANCER BASED UPON PUBLISHED RISK ASSESSMENTS FOR DIESEL EXHAUST

Harris (1983) conducted a risk assessment for diesel particulate using data from a study of London Transport Workers (Waller, 1980). Harris assumed a relative risk of lung cancer resulting from exposure to diesel particulate of the form $(1 + \theta * X)$, where X is the excess cumulative exposure to diesel emissions in $\mu g/m^3$-years, and $\theta$ is the potency of diesel particulate measured as the increase in relative risk per $\mu g/m^3$-years exposure. Harris estimated $\theta = 1.23 \times 10^{-4}$ per $\mu g/m^3$-year (s.e. = $1.86 \times 10^{-4}$). This estimate was derived from a negative study, as evidenced by the fact that the standard error exceeds the estimate.

Harris also estimated $\theta$ using a relative potency approach that utilized epidemiological data on coke oven and roofing tar workers, and data from short-term bioassays of extracts of coke oven emissions, roofing tar emissions, and diesel emissions from three automobile engines and a Caterpillar engine. The overall mean estimate of the potency obtained from extracts from the three automobile engines was $\theta = 0.35 \times 10^{-4}$ per $\mu g/m^3$-year. The estimates obtained from the Caterpillar engine ranged from $\theta = 0.01 \times 10^{-4}$ to $\theta = 0.25 \times 10^{-4}$ per $\mu g/m^3$-year.

Smith and Stayner (1990) applied a multistage time-to-tumor model (Crump and Howe, 1984) to the data from a study of rats exposed throughout life to DE (Mauderly *et al.*, 1986). They obtained estimates for the unit risk (additional lifetime risk from exposure to 1 $\mu g/m^3$) in the range of $1 \times 10^{-5}$ to $2 \times 10^{-5}$. Their estimate assumes occupational exposure from age 18 through age 65 (47 years).

To convert this estimate of lifetime risk made from the Mauderly *et al.* animal study into estimates of $\theta$, a linear dose model for the relative risk of the form

$$RR = 1 + \theta * D_{t-5},$$

was assumed, where $D_{t-5}$ is cumulative exposure through five years prior to the age, t, of observation. The lifetime risk of death from lung cancer in a person occupationally exposed to 1 $\mu g/m^3$ from age 18 through age 65 is estimated based on mortality rates in

1971 for U.S. males by five-year age intervals for total deaths and lung cancer (1971 corresponds to the midpoint of the years of follow-up of the Garshick *et al.* (1988) cohort). The rates for lung cancer were modified by multiplication by the relative risk (RR) calculated using the appropriate exposure, D. The rates for all deaths are similarly modified to account for the increase in lung cancer death rates. A life table approach (Crump and Allen, 1985) was then applied to the modified rates to calculate the lifetime probability of death from lung cancer. The additional risk of lung cancer from exposure to diesel particulate was calculated by subtracting from this the corresponding risk of death from lung cancer based on the unmodified death rates.

This procedure was applied for different values of $\theta$ until a range of values for $\theta$ were found that corresponded to the range of lifetime risks obtained from the Mauderly *et al.* study by Smith and Stayner. This range was $4.6 \times 10^{-6}$ to $9.2 \times 10^{-6}$, as listed in Table 10.

Albert and Chen (1986) applied the quantal multistage model (Crump, 1984) to pre-publication data from the same study by Mauderly *et al.* and obtained a 95% upper limit of $1.2 \times 10^{-5}$ per $\mu g/m^3$, corresponding to the exposure pattern in the animal study (seven hours per day, five days per week for life). To convert this estimate so that it corresponds to 47 years of exposure eight hours per day in humans, the following adjustment was applied:

$$(1.2 \times 10^{-5}) * (8 \text{ hours}/7 \text{ hours}) * (47 \text{ years}/75 \text{ years}) \cong 0.86 \times 10^{-5} \text{ per } \mu g/m^3$$

(see also Smith and Stayner, 1990). The corresponding estimate based on the MLE of potency from the animal data, rather than the 95% upper limit, is $0.26 \times 10^{-5}$ per $\mu g/m^3$-year. These estimates of lifetime risk were converted into estimates of $\theta$ in the same way as the estimates made by Smith and Stayner (1990). The results are displayed in Table 10.

Albert and Chen also obtained an estimate of lifetime risk based on a comparative potency method that was in the same units as the estimate obtained from the Mauderly

*et al.* data. Applying the same procedure to this estimate yielded an estimate of $\theta = 5.0 \times 10^{-6}$ per $\mu g/m^3$-year.

After reviewing epidemiological data on lung cancer from exposure to several sources of polycyclic aromatic hydrocarbons (PAHs) and short-term assay data on these extracts from these same exposure sources, Cuddihy *et al.* (1984) concluded that the "lung cancer risk from exposure to DE is not likely to result in more than 0.1 lung cancer deaths each year in a population of 100,000 people per $\mu g/m^3$." It was assumed that this risk corresponds to $7.5 \times 10^{-5}$ lifetime risk (yearly risk times 75-year life span). Using the same life table approach that was applied in Smith and Stayner's estimate, this estimate corresponds to a relative risk of $\theta = 0.46 \times 10^{-4}$ per $\mu g/m^3$-year.

# APPENDIX C

# ALTERNATIVE MODEL FOR DIESEL CANCER RISK ASSESSMENT

# APPENDIX C

# ALTERNATIVE MODEL FOR DIESEL CANCER RISK ASSESSMENT

## C.1. INTRODUCTION

As previously discussed in Chapter 11, the most appropriate method to assess cancer risk of diesel exhaust is to take into account effects of both particles (carbon core) and organics because evidence exists that both agents are involved in carcinogenic process. The reasons for this conclusion are based on the following observations: (1) organics include a variety of polycyclic aromatic hydrocarbons (PAHs) and nitroaromatics, many of which are known to be carcinogenic; (2) the results of recent studies on inert particles and carbon black in rats strongly support the hypothesis that the carbon core of the diesel particle may be the primary component responsible for the induction of lung cancer; (3) PAHs are unlikely responsible for all observed tumors because they account for less than 0.1 $\mu$g/mg particulate matter (Tong and Karasek, 1984); and (4) the observation of disproportionate high tumor incidence in high exposure animals coincides with disproportionate increase of cumulative lung burden of diesel particle as exposure concentration increases.

A workshop on Research Needs for Risk Assessment of Inhaled Particulate Matter was organized and sponsored by the U.S. Environmental Protection Agency (EPA) in March, 1992. The purpose of the workshop was to determine the extent of information that can be used for quantitative risk assessment and to discuss mechanisms of particle-induced lung tumors to serve as a guidance for future research needs. Two major, among several other, conclusions that are relevant to quantitative risk assessment were reached by the Workshop:

(1) particle overloading of the lung tissue may induce both initiation (by PAH specific adducts and adducts through oxygen radicals) and cell proliferation steps in tumor formation, and

(2) more research is needed to improve the risk assessment of particle-induced lung cancers.

Although there are not enough data available to construct a biologically based dose-response model, it is desirable to investigate implications of the hypothetical mechanisms proposed by the workshop. The purpose of the alternative modeling presented in this report is to do just that. Briefly, the biological issues and their implications to quantitative risk assessment that we would like to consider are the following.

1. Particles deposited in lung are phagocytized by alveolar macrophages. Because the phagocytizing macrophages in animals from high-dose group may be more likely to be activated to release mediators including reactive oxygen species, cytokines, and growth factors, it is of interest to determine whether or not the available tumor response data are consistent with the hypothesis that the particle burden affects both initiation and proliferation in carcinogenic process.

2. Organic materials can also induce specific adducts which may contribute to cell initiation. However, given its low content, the contribution of organics to tumor induction may be very small. Can a dose-response model that is consistent to the proposed biological concept be constructed with both organics and particles as dose metrics?

3. If a model that has the above biological interpretation and is consistent with the bioassay data can be constructed, what would be its implications on quantitative risk assessment of diesel exhaust emissions, and how would its results compare with those predicted by the linearized multistage (LMS) model?

## C.2. PRELIMINARY CONSIDERATIONS

In order to evaluate the impact of various biological assumptions on diesel risk assessment, it is necessary to construct a mathematical dose-response model that takes into account the biological mechanisms discussed in the EPA workshop. Because an issue of significant importance in diesel risk assessment is the effect of lung overloading on tumor induction, the model should possess the fol/lowing properties.

1. It should depend on both types of dose metrics: organics, and carbon core. It should allow one to account for the contribution of organics and carbon core individually and/or jointly to tumor induction/formation.

2. It should allow for the possibility that model parameters can change with time because of the increasing lung burden during exposure.

3. The cell proliferation and tumor induction/formation should be stochastic in nature; it is not realistic to assume deterministic clonal growth. For instance, it should not be required to assume that all cells divide at the same age.

To accomplish these goals, we assume that a normal cell can be initiated by both organics and carbon core. Denote the initiation rate by $\mu_1$, which is a function of background and diesel-induced rates (as specified below). Because an initiated cell (I-cell) eventually either goes into cell death, or enters the cell cycle (including cells in quiescence, $G_0$), it is reasonable to assume that the cell lifetime for an I-cell follows certain probability distribution. Under this model, a cell in $G_0$ phase is equivalent to the case where it has a very long lifetime with certain probability (i.e., in the right-hand tail of the cell lifetime distribution). At the end of an I-cell's lifetime, it either dies (death) with probability $\beta$, divides into two daughter cells (birth) with probability $\alpha$, or divides into one I-cell and one malignant cell (second transition) with probability $\mu_2$; $\alpha + \beta + \mu_2 = 1$. Instead of assuming that a single malignant cell is equivalent to a tumor as in the MVK model proposed by Moolgavarkar and colleagues (1979, 1981), we assume that a malignant cell has a certain probability to become a tumor; this probability is assumed dose-dependent, thus allowing for an evaluation of dose effect on tumor progression. It should be noted that the proposed model does not exclude the possibility that it may take more than one step (for a normal cell) to become "initiated". The rate of initiation used in the model should be viewed as a net rate which represents several genetic alterations and repairs.

## C.3. MATHEMATICAL MODEL AND PARAMETERS ESTIMATION

We shall proceed to construct a dose-response function P(t:d.D), probability of cancer by time (age) t, which depends on both organic, d, and particle (carbon core), D, and incorporates the biological concept discussed previously. Because the model parameters that are not directly observed in laboratory can only be statistically estimated from high concentration cancer bioassay data, the model constructed should not be

considered a valid model of diesel-induced carcinogenesis; uncertainty about the low-dose extrapolation still remains. Some discussions about the need for further laboratory measurements will be given later.

The model with the desirable features discussed above falls into one of several classes of stochastic models that have been developed by EPA's Office of Health and Environmental Assessment (OHEA): namely, the stochastic model which was originally proposed by Chen and Farland (1991) and extended into one with time varying parameters by Tan and Chen (1992). This model will be used as the basis for constructing a biologically based dose-response model. A brief mathematical description is presented in Appendix C-2.

The time to event data from Mauderly et al. (1987) are used to estimate model parameters. The data from Mauderly et al. are useful because they contain information on natural mortality and serial sacrifice of animals with or without (malignant) tumors, valuable information for estimating tumor latency. To utilize the information from serial sacrifice in Mauderly et al. an (E-M) algorithm is derived (see Appendix C-1) and used to calculate maximum likelihood estimates of parameters.

## C.3.1 Model Parameters and Notations

The following parameters are incorporated in the dose-response model, which includes initiation rate ($\mu_1$), proliferation rate ($\gamma\alpha$), conversion rate ($\gamma\mu_2$), and probability of progression (q). The death rate for the initiated cells is implicitly defined by $\gamma(1 - \mu_2 - \alpha)$. These parameters are all dose dependent.

D: Dose of carbon core, mg/cm$^2$ of lung epithelial surface; D varies over time

d: Dose of organics, mg/cm$^2$ of lung epithelial surface

$\mu_1$: Dose-related initiation rate (per cell per day) that depends on $\mu_0$ (background rate), d, and D by $\mu_1 = \mu_0(1 + ad + bD)$; a and b are paramaters to be estimated.

$\mu_2$: Probability of producing a malignant cell at the end of an initiated cell (I-cell) lifetime

$\alpha$: The probability that an I-cell divides into two daughter cells at the end of its lifetime

q: Probability that a single malignant cell will develop into a malignant tumor

$\gamma$: $1/\gamma$ is the mean I-cell lifetime in days; a cell lifetime ends if it either goes into mitosis, or cell death. Note that if one assumes that the probability for a cell to get into mitosis is about the same as cell death then the mean cell lifetime can be conveniently interpreted as time to mitosis (i.e., cell turnover time); thus, shorter cell lifetime implies more frequent cell division. Note that the time to mitosis is a random variable here, not a fixed constant as in the assumption made in the Greenfield et al. (1984) model that has been used extensively by Cohen and Ellwein (1988) to analyze experimental bladder cancer.

N: Number of (normal) target cells

## C.3.2. Practical Considerations

By statistical theory alone the E-M algorithm developed in this report provides an elegant procedure which can be used to test hypotheses whether a particular parameter is influenced by organics and carbon core individually or both together. For instance, one could postulate that the parameter $\gamma$ (reciprocal of which represents mean cell lifetime) is given by $\gamma(d,D_i) = \gamma_0 + \gamma_{11}d + \gamma_{12}D_i$, and then proceed to test a null hypothesis that $\gamma_{11} = 0$, no effect of organics on cell lifetime. This temptation, however, must be resisted because there would be too many parameters that must be estimated if such statistical tests are to be performed. Therefore, rather than performing such a statistical exercise, we proceed with a biologically plausible assumption that parameters q and $\gamma$ depend only on lung burden of carbon core, C.

The duration of the Mauderly et al. study was about 940 days. To construct a dose-response model with time-dependent lung burden, the time interval (0,940] is divided into five subintervals; each subinterval spans 6 mo except for the last subinterval, which spans from 730 (2 years) to 940 days. Corresponding to an ambient air concentration of diesel emissions in $mg/m^3$, the deposition-retention model developed by Yu et al. is used to calculate dosimetric $(d, D_i)$, $i = 1, 2, ..., 5$, where organics dose, d, is not changing with time because it reaches steady state quickly after exposure begins and $D_i$ is the lung burden of carbon core during the ith subinterval.

The assumptions about dose-parameters relationship are given below.

1. The initiation rate associated with a lung burden $\{d, D_i, i = 1, 2, ..., 5\}$ is given by $\mu_1(d,D_i) = \mu_0(1 + a * d + b * D_i)$, for $i = 1, 2, ..., 5$. This is the only parameter that is assumed to depend on both d and D.

2. Probability of tumor formation from a malignant cell is assumed to be dependent on lung burden D by $q(D_i) = q_0 + q_1 D_i$, $i = 1, 2, ..., 5$. To simplify calculation, the possibility that q is also dependent on organics d is not considered.

3. The cell lifetime parameter $\gamma$ is assumed related nonlinearly to lung burden D by $\gamma(D_i) = \gamma_0 + \gamma_1 \mathrm{Log}(1 + D_i)$, $i = 1, 2, ..., 5$.

To reduce the number of parameters that must be estimated from the Mauderly data, some of the background parameters ($\mu_0$, $q_0$, and $\gamma_0$) for the dose-response model are estimated from the National Toxicology Program (NTP) historical control rate on Fischer-344 rats (reconstructed from Portier et al., 1986). Giving these background parameters, the dose-related parameters are then estimated by the E-M algorithm, which is described in Appendix C-2. Using tumor response data from Mauderly et al. (1987) and the corresponding dosimetric in Table C-1, the resultant parameter estimates for the model are given in Table C-2. To have some appreciation about the implication of the Mauderly et al. (1987) study, the estimated initiation and proliferation (for I-cells) rates for the study are given in Table C-3. Although these values may not represent reality (because they are not actual laboratory measurements), they could be used as a guidance for future research planning. For instance, Table C-3 (along with a discussion about Table C-7) suggests that a slight increase of proliferation rate could cause a drastic increase on tumor incidence, but only if the initiation rate is high enough. This conclusion seems to suggest that although the promotion effect of growth factors is important for tumor induction, the initiation effect of carbon core and/or organics is also essential.

TABLE C-1. DOSIMETRIC ($mg/cm^2$ lung surface) USE IN MODELING[a]

| Exposure group | d | $D_1$ | $D_2$ | $D_3$ | $D_4$ | $D_5$ |
|---|---|---|---|---|---|---|
| 0.35 | 2.5E-6 | 6.23E-5 | 8.75E-5 | 8.97E-5 | 9.02E-5 | 9.02E-5 |
| 3.5 | 3.6E-5 | 7.54E-4 | 2.40E-3 | 3.91E-3 | 5.25E-3 | 6.29E-3 |
| 7.08 | 7.3E-5 | 1.98E-3 | 5.49E-3 | 8.56E-3 | 1.12E-2 | 1.44E-2 |

[a]d is organics; $D_i$, i = 1, 2, ..., 5, are average lung burden of carbon core over five time intervals. These values are calculated by Yu et al. retention model in Appendix D.

TABLE C-2. MAXIMUM LIKELIHOOD ESTIMATES FOR MODEL PARAMETERS

| Parameter[a] | Estimate |
|---|---|
| $\mu_0$ | 1.033E-7 |
| a | 1.103E+4 |
| b | 3.214E+2 |
| $\mu_2$ | 7.907E-7 |
| $q_0$ | 1.035E-1 |
| $q_1$ | 5.332E-2 |
| $\gamma_0$ | 1.662E-2 |
| $\gamma_1$ | 2.647 |
| $\alpha$ | 5.443E-1 |
| N[b] (given) | 8.80E+7 |

[a]Background parameters $\mu_0$, $q_0$, and $\gamma_0$ are estimated separately from NTP historical control data.
[b]The number of target cells N is assumed to be 10-fold of Type II cells in mice, which is given in Kauffman (1974). It is not essential for N to be given accurately because $N\mu_0$ appears as a single term in the model; the estimated $\mu_0$ will compensate for the under- or over-estimation of N.

TABLE C-3. RELATIVE MAGNITUDE OF INITIATION $\frac{\mu_1(d,D)}{\mu_0}$ AND PROLIFERATION ($T[D]/T_0$) POTENTIAL FOR THE EXPOSED VERSUS CONTROL GROUPS IN MAUDERLY ET AL. (1987) STUDY

| | Time Interval | Exposed Groups | | |
|---|---|---|---|---|
| | | Low | Mid | High |
| Initiation | 1 | 1.048 | 1.639 | 2.442 |
| | 2 | 1.056 | 2.168 | 3.570 |
| | 3 | 1.056 | 2.654 | 4.556 |
| | 4 | 1.056 | 3.084 | 5.405 |
| | 5 | 1.056 | 3.419 | 6.433 |
| Proliferation | 1 | 1.004 | 1.052 | 1.137 |
| | 2 | 1.006 | 1.166 | 1.379 |
| | 3 | 1.006 | 1.270 | 1.590 |
| | 4 | 1.006 | 1.362 | 1.770 |
| | 5 | 1.006 | 1.424 | 1.989 |

## C.4. RESULTS

The dose-response model predicts only probability of tumor occurrence (i.e., tumor incidence) by time t due to an exposure scenario. Because the probability of tumor occurrence is not directly observable (note that when an animal dies with a tumor, it only tells us that a tumor occurred before that time), the model can not be evaluated against the observed data. (Although it is possible to use the dose-response model, with additional assumptions, to calculate tumor mortality, we prefer to evaluate the dose-response model alone because it will be used to predict cancer risk). To evaluate the reasonableness of the model constructed, it is possible only to compare the observed tumor mortality rate (after adjusting for the competing risk) and the predicted tumor incidence rate, with the understanding that observed values are expected to be smaller (this is particularly true at an early stage of tumor development when a tumor is small) than the predicted tumor incidence. Table C-4 appears to bear this out; all of the predicted tumor incidences are either greater than the observed tumor mortality rates, or within the confidence bounds calculated from the observed tumor mortality. The observed tumor mortality rate is calculated by life-table approach. The probability of

tumor mortality can only be calculated up to about 900 days because after 900 days tumors are no longer discovered by natural mortality only; in fact, the majority of tumors are discovered by sacrifice.

## C.5. RISK PREDICTIONS UNDER VARIOUS EXPOSURE SCENARIOS

For comparison, excess lifetime risks (see Tables C-5 and C-6) due to various exposure scenarios are calculated by the alternative model and the linearized multistage (LMS) model. Both point (maximum likelihood estimate) and 95% upper bound estimates are provided for the alternative model, whereas only upper bound estimate is provided for the LMS model because its linear component (which is notoriously unstable) is estimated to be 0. The 95% upper bound for the alternative model is calculated by the same approach as for the LMS model; (i.e., by increasing parameters a and b until the log-likelihood exceeds a critical value). To extrapolate from animal-based risk estimates to human, two assumptions are made: (1) lung burden in terms of $\mu g/cm^2$ of lung surface is equally potent between animals and humans, and (2) 6 mo of animal life is equivalent to 18 years of human life. The latter assumption is necessary because life-span must be divided into five subintervals to account for different parameter values.

TABLE C-4. COMPARISON OF OBSERVED TUMOR MORTALITY RATE AND PREDICTED PROBABILITY OF CANCER OCCURRENCE BY TIME (t) WHEN A (MALIGNANT) TUMOR WAS OBSERVED IN RATS

| Exposure ($mg/m^3$) | Time Tumor Observed (days) | Observed Tumor Mortality Rate[a] (95% C. I.) | | Predicted Tumor Rate by Time (t) |
|---|---|---|---|---|
| Control | 538 | 0.0051 | (0, 0.015) | 0.002 |
| | 551 | 0.010 | (0, 0.028) | 0.003 |
| 0.35 | 710 | 0.007 | (0, 0.022) | 0.005 |
| | 863 | 0.025 | (0, 0.063) | 0.008 |
| 3.50 | 891 | 0.016 | (0, 0.126) | 0.039 |
| | 895 | 0.036 | (0, 0.052) | 0.040 |
| 7.08 | 646 | 0.006 | (0, 0.019) | 0.039 |
| | 672 | 0.013 | (0, 0.037) | 0.046 |
| | 701 | 0.021 | (0, 0.041) | 0.054 |
| | 729 | 0.027 | (0, 0.059) | 0.064 |
| | 742 | 0.039 | (0.004, 0.075) | 0.069 |
| | 798 | 0.052 | (0.009, 0.095) | 0.097 |
| | 810 | 0.066 | (0.016, 0.115) | 0.104 |
| | 839 | 0.081 | (0.023, 0.138) | 0.123 |
| | 840 | 0.096 | (0.032, 0.161) | 0.123 |
| | 847 | 0.112 | (0.041, 0.183) | 0.128 |
| | 856 | 0.129 | (0.052, 0.207) | 0.135 |
| | 859 | 0.146 | (0.063, 0.229) | 0.137 |
| | 883 | 0.168 | (0.077, 0.259) | 0.156 |
| | 895 | 0.191 | (0.091, 0.291) | 0.166 |

[a]Calculated by the life table procedure. Note that observed values are mortality, which are expected to be smaller than the (predicted tumor) incidence. This expectation is particularly true at early stage of tumor development when a tumor was small.

Table C-5 compares predicted risks for humans due to continuous exposure (24 h/day) calculated by alternative and LMS models. It is interesting to see that risk calculations under various exposure concentrations are very similar using the two

different models. Table C-6 gives excess risks due to exposure to 2.6 $\mu g/m^3$ of diesel emissions, 16 h/day, 7 days/week; and 15 $\mu g/m^3$, 8 h/day, 5 days/week. The concentration 2.6 $\mu g/m^3$ was reported by EPA's Office of Mobile Sources to be the annual mean exposure of the U.S. population to diesel particulate matter in 1986 and is only slightly higher than the most recent estimate of 2.03 $\mu g/m^3$ in an EPA draft document (Motor Vehicle-Related Air Toxic Study, April, 1993); the concentration 15 $\mu g/m^3$ was reported to be the particulate exposure for workers on urban freeways in an EPA report by Carey (Air Toxics Emissions from Motor Vehicles, 1987, EPA-AA-TSS-PA-86-5). For the general population exposed to an ambient air concentration of 2.6 $\mu g/m^3$, the risk to normal (i.e., persons with normal respiratory functions) and smokers of 20 pack-years (as defined by Bohning et al., 1982) are provided. According to Bohning et al. the retention half-life for insoluble particle increases from 296 days for persons with normal respiratory function to 519 days for persons with a smoking history of 20 pack-years. This information is used to reduce the alveolar clearance rate in the dosimetric calculations using the same retention model that is also used to calculate dosimetric in Table C-1.

The excess lifetime risks in Tables C-5 and C-6 are calculated by actuarial life-table approach using the survival probability of the NTP control animals provided in Portier et al. (1986). Conceptually, this approach can be viewed as a weighted average of the probability of cancer occurrence over entire lifetime, weighted by survival probability. This approach is more appropriate than the one used previously in the draft report in which probability of cancer occurrence at a preselected time (730 days) is used to represent the lifetime risk; it is more appropriate because tumors here occur very late in life.

TABLE C-5. COMPARISON OF EXCESS RISK FOR HUMANS DUE TO CONTINUOUS EXPOSURE OF VARIOUS CONCENTRATIONS OF DIESEL EXHAUST EMISSIONS UNDER TWO DIFFERENT MODELS

| Exposure Concentration ($\mu g/m^3$) | Alternative Model[a] | | LMS Model[b] |
|---|---|---|---|
| | MLE | 95% u.b. | |
| 0.01 | 7.68E-8 | 1.35E-7 | 1.71E-7 |
| 0.1 | 8.12E-7 | 1.41E-6 | 1.72E-6 |
| 1.0 (unit risk) | 8.16E-6 | 1.65E-5 | 1.74E-5 |
| 100 | 5.58E-4 | 9.63E-4 | 1.74E-4 |
| 1,000 | 2.60E-2 | 4.22E-2 | 3.33E-2 |

[a]MLE: maximum likelihood estimate; 95% u.b.: 95% upper bound estimate. These are calculated using the alternative dose-response model.
[b]LMS: calculated by linearized multistage model (slope = 9.04 per $mg/cm^2$ of lung surface), using carbon core as dosimetric. Only malignant tumors are used in the calculations.

TABLE C-6. EXCESS LIFETIME RISK FOR HUMANS DUE TO EXPOSURE TO DIESEL EXHAUST EMISSIONS, UNDER VARIOUS EXPOSURE SCENARIOS

| Exposure Pattern | Alternative Model[a] | | LMS[b] |
|---|---|---|---|
| | MLE | 95% u.b. | |
| 2.6 $\mu g/m^3$, 16 h/day, 7 days/week Normal person | 1.41E-5 | 2.44E-5 | 3.00E-5 |
| 2.6 $\mu g/m^3$, 16 h/day, 7 days/week 20 pack-year smoker | 2.32E-5 | 3.61E-5 | 5.38E-5 |
| 15 $\mu g/m^3$, 8 h/day, 5 days/week | 3.12E-5 | 5.17E-5 | 6.18E-5 |

[a]MLE: maximum likelihood estimate; 95% u.b.: 95% upper bound estimate. These are calculated using the alternative dose-response model.
[b]LMS: calculated by linearized multistage model, using carbon core as dosimetric. Only malignant tumors are used in the calculations.

## C.6. IMPLICATIONS OF THE ALTERNATIVE MODEL

Before proceeding to discuss implications of the alternative model on risk assessment, it should be noted that the parameters used in the model are estimated on the basis of high exposure concentration cancer bioassay data, not on the basis of data from laboratory measurements (e.g., mitotic rates for cells from normal and preneoplastic lesions measured over time), which usually can be obtained over lower range of exposure concentrations. Therefore, uncertainty associated with low-dose extrapolation still remains. For this reason, we will refrain from using the model to evaluate low-dose risk estimations, but rather to evaluate the relative contribution of each biological component (e.g., initiation by organic and carbon core, individually or jointly) in the model to cancer induction.

On the basis of the constructed alternative dose-response model, some specific inferences could be made from Table C-7 by changing parameter values of the original model. Table C-7 provides a comparison of risks calculated with changed parameters, assuming that animals are exposed to 7.08 $mg/m^3$ of diesel exhaust emissions, 7 h/day, 5 days/week (which is identical to the exposure pattern of the highest exposed group in Mauderly et al., 1987).

**TABLE C-7. EFFECT OF CHANGING DOSE-DEPENDENT INITIATION AND PROMOTION PARAMETERS**
**(Animals are assumed to be exposed to 7.08 $mg/m^3$ of diesel emissions in air, 7 h/day, 5 days/week for life [i.e., the highest exposure group in Mauderly et al., 1987].)**

| Case Number | Parameters Changed | Risk at 938 Days | Ratio to Original Model |
|---|---|---|---|
| 1 | None (original model) | 0.2067 | 1.00 |
| 2 | a = 0<br>b = 0 | 0.0663 | 0.321 |
| 3 | a = 0 | 0.1616 | 0.782 |
| 4 | b = 0 | 0.1165 | 0.564 |
| 5 | a = 2.813a[a]<br>b = 0 | 0.2007 | 0.971 |
| 6 | $\gamma_1 = 1.378\gamma_1$[a] | 0.3513 | 1.700 |
| 7 | $\gamma_1 = 1.756\gamma_1$[a] | 0.4537 | 2.195 |
| 8 | $\gamma_1 = 0$ | 0.0319 | 0.154 |

[a]a = 2.813a implies that a is increased by 2.813 times of its original value.

The following observations can be made from Table C-7.

1. When there is no diesel induced initiation (Case 2), the risk is 32% of the original model (i.e., the model without changing parameters), in contrast to 42% when exposure concentration is reduced from 7.08 to 1 $mg/m^3$ (not shown here). Therefore, the role of diesel-induced initiation in cancer induction increases with increasing exposure concentrations. This conclusion is intuitively obvious because spontaneous induction of initiated cells play a bigger role in cancer induction when concentration is lower. A practical implication of this observation is that reduction of non-diesel-induced initiation (e.g., by smoking) could have greater proportion of cancer risk reduction when diesel concentration is low than when the concentration is high.

2. Cases 3 and 4 indicate that initiation by either carbon core, or organics contributes significantly to tumor incidence.

3. Case 5, along with observation Number 2 above, suggests that although diesel-induced I-cells play an important role in cancer induction, the role of initiation, however, could be assumed by either organics or carbon core alone by increasing their respective proportions. One implication is that, although existence of I-cells are important for tumor induction, these I-cells could be induced by any agent that initiates (e.g., smoking).

4. Cases 6 to 8 suggest that a small change of proliferation parameter $\gamma$ could have a disproportionate change of cancer risk. Because this parameter is assumed a function of carbon core dose, lung burden overloading has a significant effect on cancer incidence. In the absence of better information, it is assumed in this report that carbon core continues to have effect at low doses.

These four observations together suggest that while effect of growth factors (which may increase value of $\gamma$) by particle overloading is important, the initiation effect of carbon core and/or organics is also essential. Although this conclusion is only tentative because the model parameters are estimated on the basis of high concentration bioassay data, they do suggest the importance of studying the role of carbon core and organics on initiation and promotion at low- versus high-exposure concentrations. Does the relative initiation potential between organics and carbon core differ at high and low concentrations? Along with the results in Table C-6, it also suggests that a subcohort of workers who were smokers and exposed to high concentrations of diesel exhaust for a long duration would be expected to have higher lung cancer mortality.

It is interesting to observe from Table C-8 that, under the same exposure conditions, risk is greater when exposure begins later in life. This model-based conclusion is due to the fact that older animals have more spontaneously (including non-diesel) induced initiated cells that have potential to be proliferated, converted to malignant cell, and then progressing to cancer. (Note that the above observation would not contradict any observation that might show that younger animals are more sensitive to diesel exposure than older animals if a treatment induces more initiated cells in the younger animals). Assuming that the above model-generated hypothesis is realistic, an important implication is that initiation by nondiesel agents should be considered when assessing risk to humans due to exposure to diesel emissions.

**TABLE C-8. EXCESS CANCER RISK TO RATS 200 AND 300 DAYS AFTER TERMINATION OF 6-MO EXPOSURE TO D = 7.30E-5 mg/cm$^2$ OF ORGANICS, AND D = 1.89E-3 mg/cm$^2$ OF CARBON CORE[a]**

| | Days After Exposure Terminated | |
|---|---|---|
| Exposure Period (mo) | 200 | 300 |
| >6 | 1.48E-3 | 1.96E-3 |
| 6 to 12 | 4.01E-3 | 5.25E-3 |
| 12 to 18 | 8.32E-3 | 1.05E-2 |

[a]The lung burden is assumed to be zero over unexposed periods. It may not be a realistic assumption because
the lung burden is expected to linger over the following periods after exposure terminated; however, the assumed exposure condition serves the purpose better here.

## C.7. CONCLUSIONS AND SUMMARY

1. The risk predictions by both alternative and LMS models are comparable over a range of exposure concentrations that is of practical interest. However, this conclusion is valid only under the assumption that the effect of carbon core on each biological component (e.g., initiation) in the model continues to exist at low doses (see further discussions about uncertainties below). Based on the Mauderly et al. (1987) study, the risks associated with continuous exposure to 1 $\mu g/m^3$ of diesel emissions calculated by two different models are summarized below:

| Lung Tumor Data Used | Alternative Model MLE | Alternative Model 95% u.b. | LMS Model 95% u.b. |
|---|---|---|---|
| Mlignant tumors | 8.16E-6 | 1.65E-5 | 1.74E-5 |
| Total tumors | N.A. | N.A. | 3.44E-5 (taken from Chapter 11) |

2. The model suggests that populations with higher expected background rate (e.g., smokers) may be subjected to higher lung cancer risk than the populations with lower background rate. It is noted that U.S. females have about the same background lung cancer rates as the Fischer-344 rats (about 1 to 2%), whereas U.S. males have a background rate of 6%. However, because most of lung cancers are smokers, the risk to nonsmokers (males or females) should be about the same. The use of the unit risk estimate provided in Chapter 11 may somewhat underestimate risk to smokers (or other respiratory-impaired persons) unless adjustment on lung burden is made. Table C-6 provides an example of such adjustment.

## C.8. DISCUSSIONS ABOUT UNCERTAINTIES OF RISK ESTIMATES

Although, it is interesting to note that risk estimates by the LMS model are comparable to those calculated by the alternative model, there are uncertainties about low-dose extrapolation by the alternative (as well as by the LMS) model: first, the model parameters are estimated statistically, not measured in the laboratory; and second, the model parameters are estimated on the basis of high-exposure data, the relationship between a parameter and exposure below the exposure range remains unknown, and the dose-parameter relationship used in the model may not be adequate for low-dose extrapolation. For instance, it is assumed that initiation rate is linearly related to doses of carbon core. Such an assumption needs be evaluated. The risk at low doses would be overestimated in this report if the relationship between initiation rate and carbon core is sublinear (concave upward). The sublinear assumption would be reasonable if there is no effect of initiation by carbon core dose (D) at low concentration. On the other hand, the risk would be underestimated if the relationship is supralinear. Therefore, it is important to evaluate how increase of diesel-exposure concentration affects initiation rate

over low-exposure concentrations. Similarly, it is important to know the relationship between dose of carbon core and cell (I-cells in particular) proliferation at low concentration.

Another aspect of uncertainty is the use of lung burdens (organics and carbon core) calculated by mathematical model, rather than actually measured. However, the impact of this uncertainty with regard to the conclusions reached in this report is not expected to be significantly altered even if the model-based dosimetrics are not accurate because the relative patterns of lung burdens between high- and low-exposure concentrations, and between animals and humans should be about the same. Although there is some observed total lung burden, these data are not used because of the following reasons.

1. The observed data are not separated by organics and carbon core.

2. There are no human data—these data are needed to predict risk in humans.

3. The observed data do not go beyond 730 days.

4. The desire is to be consistent with Chapter 11 so that results can be compared.

## C.9. DISCUSSIONS ABOUT FUTURE RESEARCH NEEDS

The single most important use of a biologically based dose-response model in the cancer risk assessment is to reduce uncertainty of low-dose extrapolation when the mechanism for tumor response observed at high doses differs drastically from the low doses. However, this report can focus only on the use of the model to guide future research rather than to actually reduce uncertainty of risk estimate because of our inability to obtain biological parameters in the model. If a chemical is known to induce disproportionately larger cell proliferation (in normal, initiated, and/or malignant cells) at high doses than at low doses, then a model that reflects this fact would be useful. With this in mind, our effort should be to identify "components" of carcinogenesis (e.g., increase of mitotic rate) that are disproportionately more affected at high doses than at low doses and to develop models that incorporate those high-dose effects. For the diesel risk assessment, the "components" that require further study include effects of organics

and carbon core, individually or jointly, on initiation, proliferation, conversion, and progression steps of carcinogenesis. In order to use biologically based models of carcinogenesis in risk assessments, one needs to know the relationship between parameter values in a model and exposure (or dose). Ideally, some of these parameters, if not all, should be measured directly in the laboratory, or indirectly estimating from neoplastic and preneoplastic lesions (e.g., number of foci, adenomas, and tumors in a lung).

Cell proliferation is an increase in the cell population of different stages: normal, initiated, or malignant cells. Enhanced cell proliferation of normal target cells may itself increase the frequency of mutations, either by inducing error in replication or by converting DNA adducts to mutations before DNA repair can occur. The model implies that tumor incidence is linearly proportional to initiation rate. On the other hand, enhanced cell proliferation of initiated cells could lead to more than linear increase of tumor incidence. Therefore, proliferation of I-cells has a greater impact on tumor incidence than proliferation of normal cells. However, this does not mean that initiation potential of compounds (organics or carbon core) is not important. As discussed previously, it is important to determine the ability of these compounds to initiate at low versus high doses; this has a significant implication for low-dose extrapolation. From the viewpoint of mathematical modeling, cell proliferation is the result of a decrease of cell death rate and/or an increase of mitotic rate, regardless of underlying biological mechanisms. Therefore, it is logical to construct a model (as is done here) with a proliferation component involving cell death and mitosis, and important to obtain data at the cellular level even if biological mechanism at the molecular level is not yet known. If a more precise mechanism is known and the quantitative data are available, then the proliferation component of the model can be improved by incorporating the available biological information. Most of the two-stage models consider a single malignant cell to be equivalent to a tumor. If a compound is known to affect the cell proliferation of tumor cell population, a model that incorporates tumor progression should be used. For the diesel modeling, we assume that particles could enhance the proliferation of malignant cells. This assumption needs to be verified. Another model-generated hypothesis is that persons with higher number of initiated cells are subjected to higher

lung cancer risk when exposed to diesel emissions. (A person could have a higher number of initiated cells due to exposure to diesel and/or nondiesel agents, or simply by acquiring more spontaneously induced initiated cells through aging).

In summary, information that is necessary to construct a biologically based dose-response model includes (1) identifying roles that are played by organics and carbon core (individually or jointly) with respect to initiation, proliferation, conversion, and progression, at low versus high doses; (2) quantitative measurements of cellular dynamics (e.g., mitotic rate) for cells at different stages and exposure concentrations; and (3) relationship between parameters and exposure or dose. Because many biological parameters are expected to be age-dependent, they should be measured over different time points. Furthermore, frequency and size of preneoplastic foci, nodules, and tumors could also provide useful information toward improving risk assessment. Some of these data may be obtained by initiation-promotion type of study.

# C.10. REFERENCES


Bohning D., Atkins, H., and Cohn, S. Long-term particle clearance in man: normal and impaired. Ann. Occup. Hyg. 26:259-271.

Chen, C., and Farland, W. Incorporating Cell Proliferation in Quantitative Cancer Risk Assessment: Approach, Issues, and Uncertainties. In: Chemically Induced Cell Proliferation: Implications for Risk Assessment, B. Butterworth, T. Slaga, W. Farland, and M. McClain, eds.; pp. 481-499 (Wiley-Liss, Inc., New York, 1991).

Cohen S. and Ellwein L. Cell growth dynamics in long-term bladder carcinogenesis. Toxicol. Letters, 43:151-173, 1988.

Dempster, A., Laird, N., and Rubin, D. Maximum likelihood from incomplete data via the EM algorithm. Roy. Statist. Soc., B 39:1-38, 1977.

Greenfield, R., Ellwein, L., and Cohen, S. A general probabilistic model of carcinogenic analysis experimental bladder cancer, Carcinogenesis 5:437-445, 1984.

Kauffman S. Kinetics of alveolar epithelial hyperplasia in lungs of mice exposed to urethane I. quantitative analysis of cell populations, Laboratory Investigations, 30:170-175, 1974.

S. Moolgavkar and D. Venzon, "Two-Event Models for Carcinogenesis: Incidence Curve for Childhood and Adult Tumors," Math. Biosciences 47, 55-77 (1979).

S. Moolgavkar and A. Knudson, "Mutation and Cancer: A Model for Human Carcinogenesis," Journal of the National Cancer Institute 66, 1037-1052 (1981).

Portier C, Hedges, J., and Hoel, D. Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. Cancer Research 46:4372-4378, 1986.

W. Tan and C. Chen, A nonhomogeneous stochastic model of carcinogenesis for assessing risk of environmental agents. To appear in: Mathematical Population Dynamics, Proceedings of the Third International Conference, Pau, France, 1992.

# APPENDIX C-1

# E-M ALGORITHM

The E-M algorithm is derived below. It will be used to calculate maximum likelihood estimate of parameters of the alternative model. Data used for the E-M algorithm is taken from Mauderly et al. (1987), which includes time when an animal died (natural mortality or sacrifice) with or without (malignant) tumors. The computer program for the E-M calculations was developed by Mr. Daliang Chang of the Computer Science Corporation under an EPA contract. The theory of E-M algorithm can be found in Dempster et al. (1977).

Assume that the distinct times when animals died by either natural mortality or sacrifice are $t_1 < t_2 < ... < t_m$. The observations can be classified as follows:

$a_{1x}(i)$: observed number of natural deaths without tumor at time $t_i$ in the treatment group x (There are four groups for diesel data [i.e., x = 1, 2, 3, 4.]),

$a_{2x}(i)$: observed number of natural deaths with tumor at time $t_i$ in the treatment group x,

$b_{1x}(i)$: series sacrifice at time $t_i$ without tumor in the treatment group x,

$b_{2x}(i)$: series sacrifice at time $t_i$ with tumors in the treatment group x.

Let $T_d$ represent the time an animal died and T the time a tumor developed.

$\alpha_x(i) = Pr\{T_d = t_i | T_d \geq t_i, T > t_i, x\}$ (conditional probability of death without tumor)

$\beta_x(i|u) = Pr\{T_d = t_i | T_d \geq t_i, T \epsilon (t_{u-1}, t_u], x\}$ (related to deaths with tumors)

Define,

$$A_x(i) = \prod_{j=1}^{i} [1 - \alpha_x(j)],$$

$$B_x(i|u) = \prod_{j=u}^{i} [1 - \beta_x(j|u)].$$

$$S_x(t) = \Pr\{T \geq t|x\} = \exp[-\int_{t_0}^{t} h(x)dx].$$

The function $S_x(t)$ is the probability of tumor free by time t. The exact form of the hazard function h(x) and $S_x(t)$ are given in the next section.

Let

$a_{2x}(i|u)$ = number of natural death at $t_i$ with tumor developed during $(t_{u-1}, t_u]$, in the treatment group x, u < i,

$b_{2x}(i|u)$ = number of sacrifice at $t_i$ with tumor developed during $(t_{u-1}, t_u]$, in the treatment group x, u < i,

Then

$$a_{2x}(i) = \sum_{u=1}^{i} a_{2x}(i|u), \quad b_{2x}(i) = \sum_{u=1}^{i} b_{2x}(i|u)$$

Let

$$P_x(i|u) = \frac{A_x(u)S_x(t_u)\Delta_x(t_u)B_x(i-1|u)\beta_x(i|u)}{\sum_{j=1}^{i} A_x(j)S_x(t_j)\Delta_x(t_j)B_x(i-1|j)\beta_x(i|j)},$$

and

$$Q_x(i|u) = \frac{A_x(u)S_x(t_u)\Delta_x(t_u)B_x(i|u)}{\sum_{j=1}^{i} A_x(j)S_x(t_j)\Delta_x(t_j)B_x(i|j)},$$

where

$$\Delta_x(t_i) = \frac{S_x(t_{i-1})}{S_x(t_i)} - 1.$$

Given $a_{2x}(x)$, $\{a_{2x}(i|u), u = 1, ..., i\}$, is an $(i - 1)$-dimension multinomial with parameter $\{a_{2x}(i), P_x(i|u), u = 1, ..., i\}$.

Thus,
$$E[a_{2x}(i|u)|a_{2x}(i)] = a_{2x}(i)P_x(i|u).$$

Similarly, $\{b_{2x}(i|u), u = 1, ..., i\}$, is an $(i - 1)$-dimension multinormial with parameters $\{b_{2x}(i), Q_x(i|u), u = 1, ..., i\}$, and

$$E[b_{2x}(i|u)|b_{2x}(i)] = b_{2x}(i)Q_x(i|u).$$

It can be shown that the likelihood function is proportional to

$$L = \prod_x \prod_{i=1}^{m} [S_x(t_i)]^{a_{1x}(i)+b_{1x}(i)+m_x(i)}[\Delta_x(t_i)]^{m_x(i)},$$

where

$$m_x(i) = \sum_{u=i}^{m} [a_{2x}(u|i) + b_{2x}(u|i)].$$

Let

$$R_{1x}(i) = \sum_{j=i}^{m} [a_{1x}(j) + b_{1x}(j) + m_x(j)], \text{ and}$$
$$R_{2x}(i|u) = \sum_{j=i}^{m} [a_{2x}(j|u) + b_{2x}(j|u)].$$

Let

$$\Theta = (\mu_1, \mu_2, \gamma_1, ...)$$

be a vector of parameters in function S;

$$\alpha_x = [\alpha_x(1), \alpha_x(2), ..., \alpha_x(m)], \text{ and}$$
$$\beta_x(u) = [\beta_x(1|u), \beta_x(2|u), ..., \beta_x(m|u)]$$

be vectors of parameters related to conditional probabilities of death with and without tumors. These parameters, along with those in $\theta_x$ will be estimated by the E-M algorithm described below.

**The M-step:**

Given initial values $a_{2x}(i|u)$ and $b_{2x}(i|u)$, estimate

1. $\overline{\alpha_x}(i) = a_{1x}(i)/R_{1x}(i)$ and
2. $\overline{\beta_x}(i|u) = a_{2x}(i|u)/R_{2x}(i|u)$, and
3. obtain $\overline{\theta_x}$ by maximizing the log of L.

**The E-Step:**

Given the estimated values on $\alpha_x(i)$, $\beta_x(i)$, and $\theta_x$ from the M-step, compute $P_x(i|u)$ and $Q_x(i|u)$, and obtain estimates of $a_{2x}(i|u)$ and $b_{2x}(i|u)$ by

$$\overline{a_{2x}}(i|u) = a_{2x}(i)\overline{P_x}(i|u), \text{ and}$$
$$\overline{b_{2x}}(i|u) = b_{2x}(i)\overline{Q_x}(i|u).$$

With the estimated values of $a_{2x}(i|u)$ and $b_{2x}(i|u)$ available from the E-step, go back to the M-step. Repeat the same process until estimates are stabilized.

# APPENDIX C-2
# A TUMOR GROWTH MODEL

The tumor growth model with piece-wise constant parameters is taken from Tan and Chen (1992), which is an extension of a stochastic model developed by Chen and Farland (1991). This model has a similar biological motivation as the two-stage model proposed by Greenfield et al. (1984), which has been used by Cohen and Ellwein (1988) to analyze bladder tumors. However, the two models differ from each other with respect to their mathematical formulations; the one adopted in this report is a stochastic model, whereas the other is a deterministic model and does not allow for parameters estimation because the model does not have complete mathematical expression.

Although its most general form will not be used here because of the lack of data, it is worthwhile to note that the stochastic model by Chen and Farland (1991) has two desirable features: (1) it allows for any cell growth distributions (e.g., Gompertz), rather than limiting only to the exponential distribution as in other existing models; and (2) it incorporates the birth and death of tumor cells, rather than assuming that a tumor is born once a single tumor cell occurs, an assumption made by the MVK model (a model proposed by Moolgavkar et al., 1979, 1981). Therefore, if information on cell lifetime distribution, and the progression phase of tumor development is available, a reasonably realistic model can be constructed.

For completeness of the report, a brief description of the model will be presented here. The following notations are needed for the model:

N(t): number of normal (target) cells at time t,

$\mu_1$: initiation rate, and

f(t): the probability density function for the lifetime of an initiated cell (I-cell).

For an I-cell, at the end of its lifetime it either divides (mitosis) or dies (programmed or nonprogrammed death). If it enters into mitosis, it either divides into two I-cells with probability $\alpha$, or divides into one I-cell and one malignant cell (M-cell)

with probability $\mu_2$. Note that, at the end of a cell's lifetime, the probability for the cell to die is $\beta = 1 - \alpha - \mu_2$. A similar setup (i.e., to allow for any cell lifetime distribution) can be made for an M-cell. However, we will confine ourselves to a simpler version assuming that an M-cell lifetime follows an exponential distribution. Thus, we can simply assume that an M-cell follows a simple birth-death process; it can either divide into two M-cells with a rate $\alpha_m$ or die with a rate $\beta_m$.

When parameters are constant over time (ages), the hazard function is given by

$$h(t) = \mu_1\mu_2\int_0^t N(s)m(t - s)\, ds$$

where

$$m(t) = \frac{(y_2 - y_1)^2\exp[A(t)\alpha(y_2 - y_1)]}{\{(1 - y_1) + (y_2 - 1)\exp[A(t)\alpha(y_2 - y_1)]\}^2};$$

where $y_1 < y_2$ are two real roots of $\alpha y^2 - (\alpha + \beta + \mu_2 q)y + \beta = 0$; $\alpha + \beta + \mu_2 = 1$; $q = 1 - \beta_m/\alpha_m$; $A(t) = \int_0^t a(x)dx$, where $a(t) = f(t)/[1 - F(t)]$ is the hazard function of the cell lifetime, $F(t)$ is the cumulative function of $f(t)$. Two special cases of interest here are $a(t) = \gamma$, when the exponential distribution is assumed; and $a(t) = \exp(-\gamma t)$, when the Gompertz distribution is assumed.

When exponential distribution (i.e., $a(t) = \gamma$, or $A(t) = \gamma t$) and $q = 1$ are assumed, the model is equivalent to the MVK model. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed (i.e., $A(t) = [1 \quad \exp(\quad t)]/\ )$.

For the model with time-dependent parameters, assume that the study begins at time $t_0$. Divide time scale $(t_0, t]$ into k subintervals $L_j = (t_{j-1}, t_j]$, $j = 1, 2, \ldots k-1$ and $L_k = (t_{k-1}, t_k]$ where $t_k = t$ (note that these subintervals may not be the same subintervals defined by deaths or sacrifice before). The parameters that vary over subintervals $(t_{i-1}, t_i]$, $i = 1, 2, \ldots, k$ are $\mu_{1j}$, $\alpha_j$, $\beta_j$, $\mu_{2j}$, $N_j$, and those parameters related to $f(t)$. The hazard function is given by

$$h(t) = \sum_{j=1}^{k}[\mu_{1j}\mu_{2j}N_j\int_{t_{j-1}}^{t_j} m_j(t_j - s)ds]\prod_{i=j+1}^{k} m_j(t_i - t_{i-1}),$$

where

$$\prod_{i=j+1}^{k} m_j(t_j - t_{j-1}) = 1, \text{ when } j = k$$

and

$$m_j(t) = \frac{(y_{2j} - y_{1j})^2 \exp[A_j(t)\alpha_j(y_{2j} - y_{1j})]}{((1 - y_{1j}) + (y_{2j} - 1)\exp[A_j(t)\alpha_j(y_{2j} - y_{1j})])^2},$$

where $y_{1j} < y_{2j}$ are two real roots of $\alpha_j y^2 - (\alpha_j + \beta_j + \mu_{2j}q_j)y + \beta_j = 0$; $\alpha_j + \beta_j + \mu_{2j} = 1$; $q_j = 1 - \beta_{mj}/\alpha_{mj}$, $j = 1, 2, ..., k$.

When exponential distribution (i.e., $A_j(t) = \gamma_j t$ and $q_j = 1$ are assumed, the model is equivalent to the MVK model with piece-wise constant parameters. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed (i.e., when $A_j(t) = \{1 - \exp[-\gamma_j t]\}/\gamma_j$).

For the diesel alternative model, the total time is divided into five (i.e., $k = 5$) subintervals. It is shown in Tan and Chen (1992) that, under the assumption of exponential cell lifetime distribution, the tumor free distribution function, $S_x(t)$, can be written as

$$S(t) = \exp\{-\sum_{j=1}^{k} [A_{jj}(t_{j-1}, s_j) + \sum_{i=1}^{j-1} A_{ij}(t_{i-1}, s_j)]\}$$

where $s_j = t_j$ if $j < k$ and $s_j = t$ if $j = k$, and

$$A_{ij}(t_{j-1}, s_j) = 2N_j\mu_{1j}\mu_{2j}\frac{1}{w_I + z_I}\{-(s_j - t_{j-1}) + \frac{2}{\gamma_{Ij}(w_I - z_I)}\log[1 + \frac{w_I - z_I}{2w_I}(e^{w_I\gamma_{Ij}(s_j - t_{j-1})} - 1)]\}$$

$$A_{ij}(t_{i-1},s_j) = 4N_i\mu_{1i}\mu_{2j}\left[\frac{1}{\gamma_{II}(w_I^2 - z_I^2)}\right]x$$
$$\left\{\log\left[\frac{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i+1,j-1}(t_i,t_{j-1})}{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i,j-1}(t_{i-1},t_{j-1})}\right]\right.$$
$$\left.-\log\left[\frac{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{i+1,j}(t_i,s_j)}{w_I + z_I + (w_I - z_I)\exp(w_I\Delta_{ij}(t_{i-1},s_j)}\right]\right\},$$

where,

$W_I = [(\alpha + \beta + \mu_2 q)^2 - 4\alpha\beta]^{1/2}$,

$Z_I = \alpha - \beta - \mu_2 q$, and

$\Delta_{ii}(s,t) = \gamma_i(t - s)$ if both s and t are in the same closed subinterval $[t_{i-1}, t_i]$ and

$$\Delta_{ij}(s,t) = \gamma_i(t_i - s) + \sum_{r=i+1}^{j-1} \gamma_r(t_r - t_{r-1})$$

if $s \epsilon L_i$, $t \epsilon L_j$ with $t_i < t_j$

# APPENDIX D

# MODELS FOR CALCULATING LUNG BURDENS

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## D.1. INTRODUCTION

As discussed in Chapter 4 the lung burden of diesel exhaust particles (DEPs) during exposure is determined by both the amount and site of particle deposition in the lung and, subsequently, by rates of translocation and clearance from the deposition sites. Mathematical models have often been used to complement experimental studies in estimating the lung burdens of inhaled particles in different species under different exposure conditions. This section presents a mathematical model that simulates the deposition and clearance of DEPs in the lungs of rats and humans.

Diesel particles are aggregates formed from primary spheres of 15-30 nm in diameter. The aggregates are irregularly shaped and range in size from a few molecular diameters to tens of microns. The mass median aerodynamic diameter (MMAD) of the aggregates is approximately 0.2 μm. The primary sphere consists of a carbonaceous core (soot) on which numerous kinds of organic compounds are adsorbed. The organics normally account for 10% to 30% of the particle mass. However, the exact size distribution of DEPs and the specific composition of the adsorbed organics depend upon many factors, including engine design, fuels used, engine operating conditions, and the thermodynamic process of exhaust. The physical and chemical characteristics of DEPs have been reviewed extensively by Amann and Siegla (1982) and Schuetzle (1983).

Four mechanisms deposit diesel particles within the respiratory tract during exposure: impaction, sedimentation, interception, and diffusion. The contribution from each mechanism to deposition, however, depends upon lung structure and size, the breathing condition of the subject, and particle size distribution. Under normal breathing conditions, diffusion is found to be the most dominant mechanism. The other three mechanisms play only a minor role.

Once DEPs are deposited in the respiratory tract, both the carbonaceous cores and the adsorbed organics of the particles will be removed from the deposition sites as described in

Chapter 4. There are two mechanisms which facilitate this removal: (a) mechanical clearance, provided by mucociliary transport in the ciliated conducting airways as well as macrophage phagocytosis and migration in the nonciliated airways, and (b) clearance by dissolution. Since the carbonaceous soot of DEPs is insoluble, it is removed from the lung primarily by mechanical clearance, whereas the adsorbed organics are removed principally by dissolution.

## D.2. PARTICLE MODEL

To develop a mathematical model which simulates the deposition and clearance of DEPs in the lung, an appropriate particle model characterizing a diesel particle must first be introduced. For the deposition study, we employed an equivalent sphere model for the diesel particle developed by Yu and Xu (1987) to simulate the dynamics and deposition of DEPs in the respiratory tract by various deposition mechanisms. For the clearance study, we assume that a diesel particle is composed of three different material components according to their characteristic clearance rates: (1) a carbonaceous core of approximately 80 percent of the particle mass, (2) absorbed organics of about 10 percent of particle mass, and that are slowly cleared from the lung (3) adsorbed organics quickly cleared from the lung accounting for the remaining 10 percent of particle mass. The presence of two discrete organic phases in the particle model is suggested by observations that the removal of particle-associated organics from the lung exhibits a biphasic clearance curve (Sun et al., 1984; Bond et al., 1986) as discussed in Chapter 4. This curve represents two major kinetic clearance phenomena: a fast phase organic washout with a half-time of a few hours and a slow phase with a half-time that is a few hundred times longer. The detailed components involved in each phase of the clearance are not known. It is possible that the fast phase consists of organics which are leached out primarily by diffusion mechanisms while the slow phase might include any or all of the following components: (a) organics which are "loosened" before they are released, (b) organics which have become intercalated in the carbon core and where release is thus impeded, (c) organics which are associated for longer periods of time due to hydrophobic interaction with other organic phase materials, (d) organics which have been ingested by macrophages and as a result effectively remain in the lung for a longer period of time due to metabolism by the macrophage; metabolites formed may interact with other cellular compo-

nents, and (e) organics which have directly acted on cellular components such as the formation of covalent bonds with DNA and other biological macromolecules to form adducts.

The above distinction of the organic components is largely mechanistic and it does not specifically imply the actual component nature of the organics adsorbed on the carbonaceous core; however, the distinction is made to account for the biphasic clearance of DEPs. However, this distinction is necessary in appreciating the dual phase nature of DEPs. For aerosols made of pure organics such as benzo(a)pyrene (BaP) and nitropyrene (NP) in the same size range of DEPs, Sun et al. (1984) and Bond et al. (1986) observed a nearly monophasic clearance curve. This might be explained by the absence of intercalative phenomena (a) and of hydrophobic interaction imposed by a heterogeneous mixture of organics (b). The measurement of a pure organic might also neglect that quantity which has become intracellular (c) or covalently bound (d).

## D.3. COMPARTMENTAL LUNG MODEL

To study the transport and removal of DEPs from the lungs, we used a compartmental model consisting of four anatomical compartments: the nasopharyngeal or head (H), tracheobronchial (T), alveolar (A), and lung associated lymph node (L) compartments as shown in Figure D-1. In addition, we used two outside compartments B and G representing, respectively, the blood and gastrointestinal (GI) tract. The alveolar compartment in the model is obviously the most important compartment for long-term retention studies. However, for short-term consideration, retentions in other lung compartments may also be significant. The presence of these lung compartments and the two outside compartments in the model therefore provides a complete description of all clearance processes involved.

In Figure D-1, $r_H^{(i)}$, $r_T^{(i)}$, and $r_A^{(i)}$ are, respectively, the mass deposition rates of DEP material component i (i=1 (core), 2 (slowly cleared organics), and 3 (rapidly cleared organics)) in the head, tracheobronchial and alveolar compartments; and $\lambda_{XY}^{(i)}$

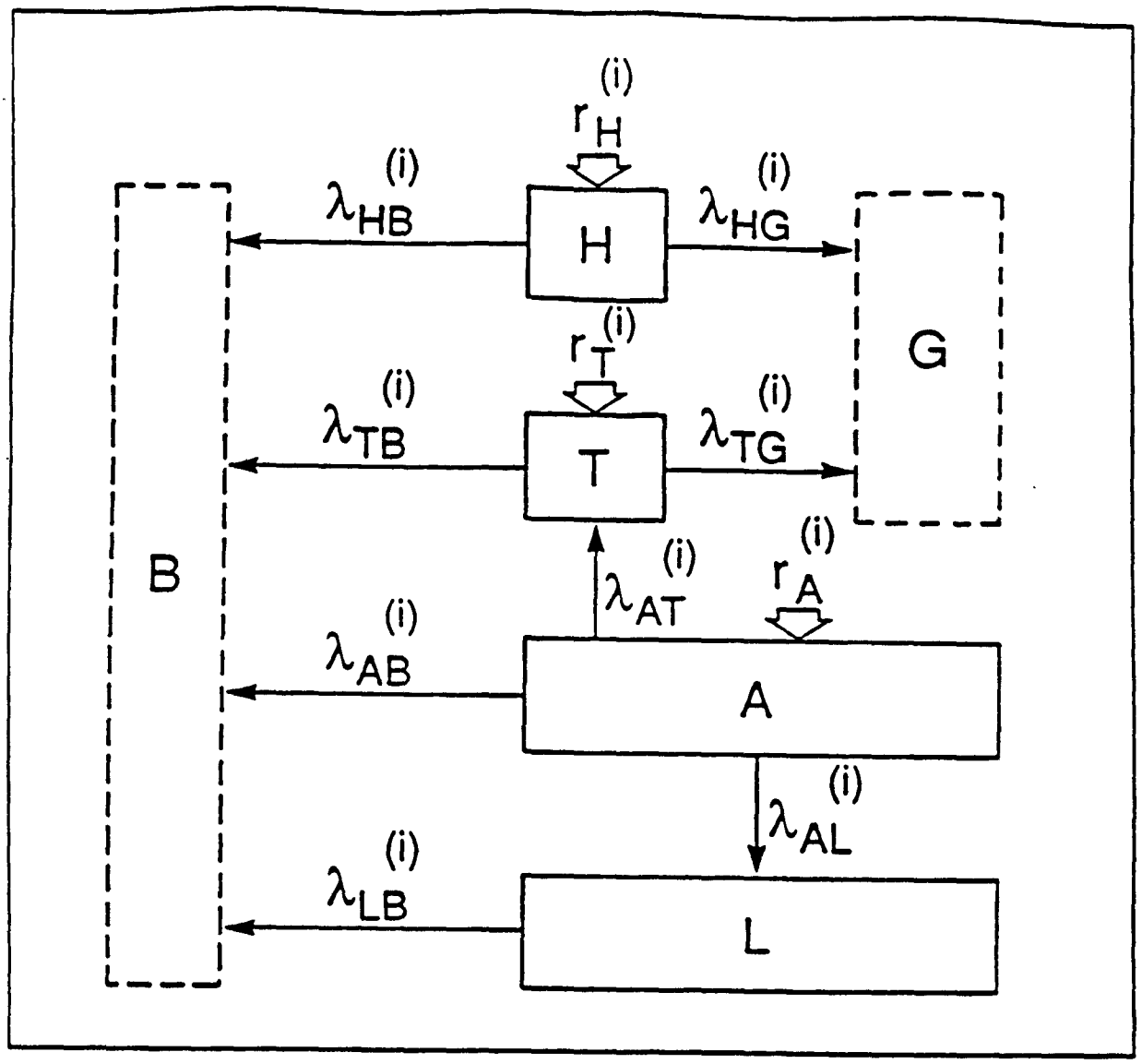


Figure D-1 Compartmental model of DEP retention.

represents the transport rate of material component i from any compartment X to any compartment Y. Let the mass fraction of material component i of a diesel particle be $fi$. Then

$$r_H^{(i)} = f_i\, r_H \quad , \tag{D-1}$$

$$r_T^{(i)} = f_i\, r_T \quad , \tag{D-2}$$

$$r_A^{(i)} = f_i\, r_A \quad , \tag{D-3}$$

where $r_H$ , $r_T$ , and $r_A$ are, respectively, the total mass deposition rates of DEPs in the H, T, and A compartments, determined from the equations:

$$r_H = c(TV)(RF)(DF)_H \quad , \tag{D-4}$$

$$r_T = c(TV)(RF)(DF)_T \quad , \tag{D-5}$$

$$r_A = c(TV)(RF)(DF)_A \quad . \tag{D-6}$$

In equations D-4 to D-6, c is the mass concentration of DEPs in the air, TV is the tidal volume, RF is the respiratory frequency, and $(DF)_H$, $(DF)_T$, and $(DF)_A$ are, respectively, the deposition fractions of DEPs in the H, T, and A compartments over a respiratory cycle. The values of $(DF)_H$, $(DF)_T$, and $(DF)_A$ which vary with the particle size, breathing conditions and lung architecture were determined from our deposition model (Yu and Xu, 1987).

The differential equations for $m_{XY}^{(i)}$ , the mass of material component i in compartment X, as a function of exposure time t can be written as

Head (H)

$$\frac{dm_H^{(i)}}{dt} = r_H^{(i)} - \lambda_{HG}^{(i)} m_H^{(i)} - \lambda_{HB}^{(i)} m_H^{(i)} \quad , \tag{D-7}$$

Tracheobronchial (T)

$$\frac{dm_T^{(i)}}{dt} = r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{TG}^{(i)} m_T^{(i)} - \lambda_{TB}^{(i)} m_T^{(i)} \quad , \tag{D-8}$$

Alveolar (A)

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_{AT}^{(i)} m_A^{(i)} - \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{AB}^{(i)} m_A^{(i)} \quad , \tag{D-9}$$

Lymph Nodes (L)

$$\frac{dm_L^{(i)}}{dt} = \lambda_{AL}^{(i)} m_A^{(i)} - \lambda_{LB}^{(i)} m_L^{(i)} \quad . \tag{D-10}$$

Equation D-9 may also be written as

$$\frac{dm_A^{(i)}}{dt} = r_A^{(i)} - \lambda_A^{(i)} m_A^{(i)} \quad , \tag{D-11}$$

where

$$\lambda_A^{(i)} = \lambda_{AT}^{(i)} + \lambda_{AL}^{(i)} + \lambda_{AB}^{(i)} \quad . \tag{D-12}$$

is the total clearance rate of material component i from the alveolar compartment. In equations D-7 to D-10, we have assumed vanishing material concentration in the blood compartment to calculate diffusion transport.

The total mass of the particle-associated organics in compartment X is the sum of $m_X^{(2)}$ and $m_X^{(3)}$ the total mass of DEPs in compartment X is equal to

$$m_X = m_X^{(1)} + m_X^{(2)} + m_X^{(3)} \tag{D-13}$$

The lung burdens of diesel soot (core) and organics are defined, respectively, as

$$m_{Lung}^{(1)} = m_T^{(1)} + m_A^{(1)} \quad , \tag{D-14}$$

and

$$m_{Lung}^{(2)+(3)} = m_T^{(2)} + m_A^{(2)} + m_T^{(3)} + m_A^{(3)} \quad . \tag{D-15}$$

Because the clearance of diesel soot from compartment T is much faster than from compartment A, $m_T^{(1)} \ll m_A^{(1)}$ a short time after exposure, equation D-14 leads to

$$m_{Lung}^{(1)} \cong m_A^{(1)} \quad . \tag{D-16}$$

Solution to equations D-7 to D-10 can be obtained once all the transport rates $\lambda_{XY}^{(i)}$ are known. When $\lambda_{XY}^{(i)}$ are constant, which is the case of linear kinetics, equations D-7 to E-10 will have a solution that increases with time at the beginning of exposure but eventually saturates and reaches a steady-state value. This is the classical retention model developed by the International Eommission of Radiological Protection (ICRP, 1979). However, as discussed in Chapter 4, data have shown that when rats are exposed to DEPs at high concentration for a prolonged period, the diesel soot accumulates in various peribronchial and subpleural regions in the lung and the long-termed clearance is impaired. This is the so-called overload effect, observed also for other insoluble particles. The overload effect cannot be predicted by the classical ICRP model. Soderholm (1981) and Strom et al. (1987, 1988) have proposed a model to simulate this effect by adding a separate sequestrum compartment in the alveolar region. In the present approach, a single compartment for the alveolar region of the lung is used and the overload effect is accounted for by a set of variable transport rates $\lambda_{AT}^{(i)}$, $\lambda_{AL}^{(i)}$, and $\lambda_A^{(i)}$ which are functions of $m_A$. The transport rates $\lambda_A^{(i)}$ and $\lambda_{AL}^{(i)}$ in equations D-7 to E-10 can be determined directly from experimental data on lung and lymph node burdens, and $\lambda_{AT}^{(i)}$ and $\lambda_{AB}^{(i)}$ from equation D-12.

## D.4. SOLUTIONS TO KINETIC EQUATIONS

Equation D-11 is a nonlinear differential equation of $m_A^{(i)}$ with known function of $\lambda_A^{(i)}$. For diesel soot, this equation becomes

$$\frac{dm_A^{(1)}}{dt} = r_A^{(1)} - \lambda_A^{(1)}(m_A)m_A^{(1)} \quad . \tag{D-17}$$

Because clearance of the particle-associated organics is much faster than diesel soot, $m_A^{(2)}$ and $m_A^{(3)}$ constitute only a very small fraction of the total particle mass (less than one percent) after a long exposure and we may consider $\lambda_A^{(1)}$ as a function of $m_A^{(1)}$ alone. Equation D-17 is then reduced to a differential equation with $m_A^{(1)}$ the only dependent variable.

The general solution to equation D-17 for constant $r_A^{(1)}$ at any time, t, can be obtained by the separation of variables to give

$$\int_0^{m_A^{(1)}} \frac{dm_A^{(1)}}{r_A^{(1)} - \lambda_A^{(1)} m_A^{(1)}} = t \quad . \tag{D-18}$$

If $r_A^{(1)}$ is an arbitrary function of t, equation D-17 needs to be solved numerically such as by a Runge-Kutta method. Once $m_A^{(1)}$ is found, the other kinetic equations D-7 to D-10 for both diesel soot and the particle-associated organics can be solved readily, since they are linear equations. The solutions to these equations for constant $r_H^{(i)}$, $r_T^{(i)}$, and $r_A^{(i)}$ are given below:

Head (H)

$$m_H^{(i)} = r_H^{(i)}/\lambda_H^{(i)} + (m_{H0}^{(i)} - r_H^{(i)}/\lambda_H^{(i)}) \exp(-\lambda_H^{(i)} t) \tag{D-19}$$

$$where \quad \lambda_H^{(i)} = \lambda_{HG}^{(i)} + \lambda_{HB}^{(i)} \tag{D-20}$$

Tracheobronchial (T)

$$m_T^{(i)} = \exp(-\lambda_T^{(i)} t) \int_0^t ( r_T^{(i)} + \lambda_{AT}^{(i)} m_A^{(i)} ) \exp(\lambda_{AT}^{(i)} t) \, dt + m_{T0}^{(i)} \tag{D-21}$$

$$where \quad \lambda_T^{(i)} = \lambda_{TG}^{(i)} + \lambda_{TB}^{(i)} \tag{D-22}$$

Lymph Nodes (L)

$$m_L^{(i)} = \exp(-\lambda_{LB}^{(i)} t) \int_0^t \lambda_{AL}^{(i)} m_A^{(i)} \exp(\lambda_{LB}^{(i)}) \, dt + m_{L0}^{(i)}) \tag{D-23}$$

In equations D-19 to D-23, $m_{XO}^{(i)}$ represents the value of $m_X^{(i)}$ at t = 0.

In the sections to follow, the methods of determining $r_H^{(i)}$, $r_T^{(i)}$, and $r_A^{(i)}$, or $(DF)_H$, $(DF)_T$, and $(DF_A$ $r_H^{(DF)}$, $r_T^{(DF)}$, and $r_A^{(DF)}$ as well as the values of $\lambda_{XY}^{(i)}$ in the compartmental lung model are presented.

## D.5. DETERMINATION OF DEPOSITION FRACTIONS

The mathematical models for determining the deposition fractions of DEPs in various regions of the respiratory tract have been developed by Yu and Xu (1986, 1987) and are adopted in this report. Yu and Xu consider DEPs as a polydisperse aerosol with a specified mass median aerodynamic diameter (MMAD) and geometrical standard deviation $\sigma_g$. Each diesel particle is represented by a cluster-shaped aggregate within a spherical envelope of diameter $d_e$. The envelope diameter $d_e$ is related to the aerodynamic diameter of the particle by the relation

$$\frac{d_e}{d_a} = \phi^{-1/2}\left(\frac{C_a}{C_e}\right)^{1/2)}\left(\frac{\zeta}{\zeta_o}\right)^{1/2} \qquad \text{(D-24)}$$

where $\zeta$ is the bulk density of the particle in g/cm$^3$, $\zeta_0$ = 1 g/cm$^3$; $\phi$ is the packing density, which is the ratio of the space actually occupied by primary particles in the envelope to the overall envelope volume; and $C_x$ is the slip factor given by the expression:

$$C_x = 1 + 2\frac{\lambda}{d_x}\left[1.257 + 0.4 \exp -\left(\frac{0.55 d_x}{\lambda}\right)\right] \qquad \text{(D-25)}$$

in which $\lambda \cong 8 \times 10^{-6}$cm$^3$ is the mean free path of air molecules at standard conditions. In the diesel particle model of Yu and Xu (1986), $\zeta$ has a value of 1.5 g/cm$^3$ and a $\phi$ value of 0.3 is chosen based upon the best experimental estimates. As a result, Equation D-24 gives $d_e/d_a$ = 1.35. In determining the deposition fraction of DEPs, $d_e$ is used for diffusion and interception according to the particle model.

## D.5.1. DETERMINATION OF $(DF)_H$

Particle deposition in the naso- or oro-pharyngeal region is referred to as head or extra-thoracic deposition. The amount of particles that enters the lung depends upon the breathing mode. Normally, more particles are collected via the nasal route than the oral route because of the nasal hairs and the more complex air passages of the nose. Since the residence time of diesel particles in the head region during inhalation is very small (about 0.1 second for human adults at normal breathing), diffusional deposition is insignificant and the major deposition mechanism is impaction. The following empirical formulas derived by Yu et al. (1981) for human adults are adopted for deposition prediction of DEPs:

For mouth breathing:

$$(DF)_{H,\ in} = 0, \qquad for\ d_a^2 \leq 3000 \tag{D-26}$$

$$(DF)_{H,\ in} = -1.117 + 0.324\ \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \tag{D-27}$$

$$(DF)_{H,\ ex} = 0, \tag{D-28}$$

and for nose breathing:

$$(DF)_{H,\ in} = -0.014 + 0.023\ \log(d_a^2 Q),\ for\ d_a^2 Q \leq 337 \tag{D-29}$$

$$(DF)_{H,\ in} = -0.959 + 0.397\ \log(d_a^2 Q),\ for\ d_a^2 Q > 337 \tag{D-30}$$

$$(DF)_{H,\ ex} = 0.003 + 0.033\ \log(d_a^2 Q),\ for\ d_a^2 Q \leq 215 \tag{D-31}$$

$$(DF)_{H,\ ex} = -0.851 + 0.399\ \log(d_a^2 Q),\ for\ d_a^2 Q > 215 \tag{D-32}$$

where $(DF)_H$ is the deposition efficiency in the head, the subscripts in and ex denote inspiration and expiration, respectively, $d_a$ is the particle aerodynamic diameter in μm, and Q is the air flowrate in $cm^3$/sec.

Formulas to calculate deposition of diesel particles in the head region of children are derived from those for adults using the theory of similarity, which assumes that the air passage in the head region is geometrically similar for all ages and that the deposition process is characterized by the Stokes number of the particle. Thus, the set of empirical equations from D-26 through D-32 are transformed into the following form:

For mouth breathing:

$$(DF)_{H,\ in} = 0, \qquad for\ d_a^2 Q \leq 3000 \tag{D-33}$$

$$(DF)_{H,\ in} = -1.117 + 0.972\ logK + 0.324\ \log(d_a^2 Q),\ for\ d_a^2 Q > 3000 \tag{D-34}$$

$$(DF)_{H,\ ex} = 0. \tag{D-35}$$

and for nose breathing:

$$(DF)_{H,\ in} = -\ 0.014 + 0.690\ \log K + 0.023\ \log(d_a^2 Q),\ for\ d_a^2 Q \leq 337 \tag{D-36}$$

$$(DF)_{H,\ in} = -0.959 + 1.191\ \log K + 0.397\ \log\ (d_a^2 Q),\ \text{for}\ d_a^2 Q > 337 \tag{D-37}$$

$$(DF)_{H,\ ex} = 0.003 + 0.099\ \log K + 0.033\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q \leq 215 \tag{D-38}$$

$$(DF)_{H,\ ex} = 0.851 + 1.197\ \log K + 0.399\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q > 215 \tag{D-39}$$

where K is the ratio of the linear dimension of the air passages in the head region of adults to that of children, which is assumed to be the same as the ratio of adult/child tracheal diameters.

For rats, the following empirical equations are used for deposition prediction of DEPs in the nose:

$$(DF)_{H,\ in} = (DF)_{H,\ ex} = 0.046 + 0.009\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q \leq 13.33 \tag{D-40}$$

$$(DF)_{H,\ in} = (DF)_{H,\ ex} = -0.522 + 0.514\ \log(d_a^2 Q),\ \text{for}\ d_a^2 Q > 13.33 \tag{D-41}$$

## D.4.2. Determination of $(DF)_T$ and $(DF)_A$

The deposition model adopted for DEPs is the one previously developed for monodisperse and (Yu, 1978) and polydisperse spherical aerosols (Diu and Yu, 1983). In the model, the branching airways are viewed as a chamber model shaped like a trumpet (Figure D-2). The cross-sectional area of the chamber varies with airway depth, x, measured from the beginning of the trachea. At the last portion of the trumpet, additional cross-sectional area is present to account for the alveolar volume per unit length of the airways.

Inhaled diesel particles that escape capture in the head during inspiration will enter the trachea and subsequently the bronchial airways (compartment T) and alveolar spaces (compartment A).

Assuming that the airways expand and contract uniformly during breathing, the equation for the conservation of particles takes the form:

$$\beta(A_1 + A_2)\frac{\partial c}{\partial x} + Q\frac{\partial c}{\partial x} = -Qc\eta \qquad \text{(D-42)}$$

where c is the mean particle concentration at a given x and time t; $A_1$ and $A_2$ are, respectively, the summed cross-sectional area (or volume per unit length) of the airways and alveoli at rest; $\eta$ is the particle uptake efficiency per unit length of the airway; $\beta$ is an expansion factor, given by:

$$\beta = 1 + \frac{V_t}{V_l} \qquad \text{(D-43)}$$

and Q is the air flow rate, varying with x and t according to the relation

$$\frac{Q}{Q_o} = 1 - \frac{V_x}{V_l} \qquad \text{(D-44)}$$

where $Q_0$ is the air flow rate at x = 0. In Equations D-43 and D-44, $V_t$ is the volume of new air in the lungs and $V_x$ and $V_\ell$ are, respectively, the accumulated airway volume from x = 0 to x, and total airway volume at rest.

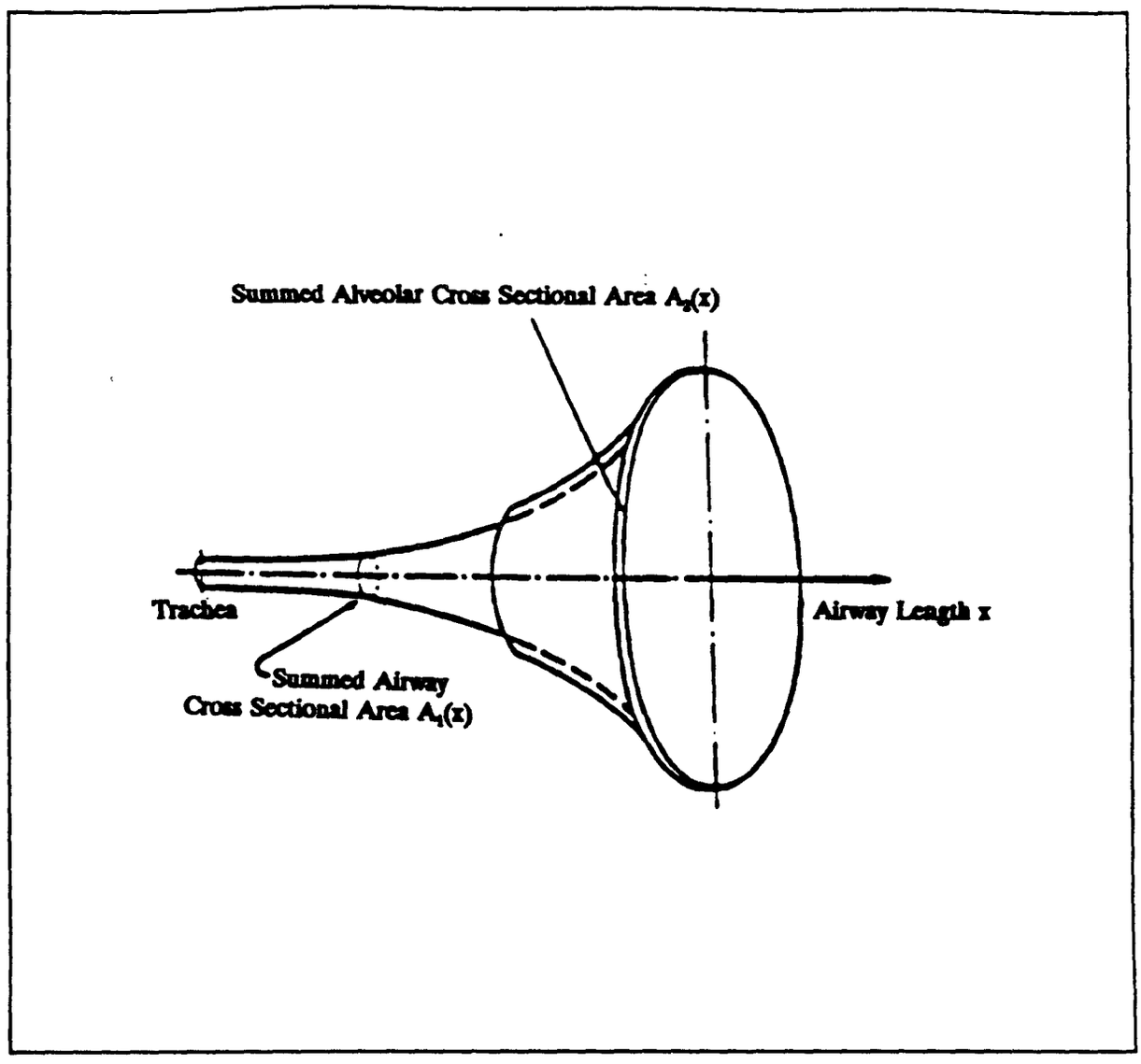


Figure D-2.Trumpet model of lung airways.

Equation D-42 is solved using the method of characteristics with appropriate initial and boundary conditions. The amount of particles deposited between location $x_1$ and $x_2$ from time $t_1$ to $t_2$ can then be found from the expression

$$DF = \int_{t_1}^{t_2} \int_{x_1}^{x_2} Qc\eta dx dt \tag{D-45}$$

For diesel particles, $\eta$ is the sum of those due to the individual deposition mechanisms described above, i.e.,

$$\eta = \eta_I + \eta_S + \eta_p + \eta_D \tag{D-46}$$

where $\eta_I$, $\eta_S$, $\eta_P$, and $_D$ are, respectively, the deposition efficiencies per unit length of the airway due to impaction, sedimentation, interception, and diffusion. On the basis of the particle model described above, the expressions for $\eta_I$, $\eta_S$, $\eta_P$, and $\eta_D$ are obtained in the following form:

$$\eta_I = \frac{0.768}{L}(St)\theta. \tag{D-47}$$

$$\eta_S = \frac{2}{\pi L}[2\varepsilon \sqrt{1 - \varepsilon^{(2/3)}} - \varepsilon^{1/3}\sqrt{1 - \varepsilon^{2/3}} + \sin^{-1} \varepsilon^{1/3}] \tag{D-48}$$

$$\eta_P = \frac{4}{3\pi L}(\Gamma - \frac{\Gamma^3}{32}) \tag{D-49}$$

$$\begin{aligned} \eta_D = \frac{1}{L}[1-0.819\exp(-14.63\Delta) - \\ 0.0976 \exp(-89.22\Delta) - \\ 0.0325 \exp(-228\Delta) - 0.0509 \exp(-125\Delta^{2/3})] \end{aligned} \tag{D-50}$$

for Reynolds numbers of the flow smaller than 2000, and

$$\eta_D = \frac{4}{L}\Delta^{1/2} (1 - 0.444\Delta^{1/2}) \tag{D-51}$$

for Reynolds numbers greater than or equal to 2000, where $ST=d_a^2u/(18\mu R)$ is the particle Stokes number, $\theta = L/(8R)$, $\in = 3\mu u_s L/(32uR)$, $\Gamma = d_e/R$, and $\Delta = DL/(4R^2u)$. In the above definitions u is the air velocity in the airway; $\mu$ is the air viscosity; L and R are, respectively, the length and radius of the airway; $u_s = C_a d^2_d/(18\mu)$ is the particle settling velocity; and $D = C_e kT(3\pi\mu d_e)$ is the diffusion coefficient with k denoting the Boltzmann constant and T the absolute temperature. In the deposition model, it is also assumed that $\eta_I$ and $\eta_P = 0$ for expiration, while $\eta_D$ and $\eta_S$ have the same expressions for both inspiration and expiration.

During the pause, only diffusion and sedimentation are present. The combined deposition efficiency in the airway, E, is equal to:

$$E = 1 - (1 - E_S)(1 - E_D) \quad . \tag{D-52}$$

where $E_D$ and $E_S$ are, respectively, the deposition efficiencies due to the individual mechanisms of diffusion and sedimentation over the pause period. The expression for $E_D$ and $E_s$ are given by

$$E_D = 1 - \sum_{i=1}^{3} \frac{4}{\alpha_i} \exp(-\alpha_i^2 \tau_D)(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2}) \exp\left[ - \frac{4\tau_D^{1/2}}{\pi^{1/2}(1 - \sum_{i=1}^{3} \frac{4}{\alpha_i^2})} \right] \tag{D-53}$$

where $\tau_D = D\tau/R^2$ in which $\tau$ is the pause time and $\alpha_1$, $\alpha_2$, and $\alpha_3$ are the first three roots of the equation:

$$J_o(\alpha) = 0 \quad . \tag{D-54}$$

in which $J_o$ is the Bessel function of the zeroth order, and:

$$E_S = 1.1094\tau_S - 0.1604\tau_S^2, \; for \; 0 < \tau_S \leq 1. \tag{D-55}$$

and

$$E_S = 1 - 0.0069\tau_S^{-1} - 0.0859\tau_S^{-2} - 0.0582\tau_S^{-3}, \quad \textit{for } \tau_S > 1, \tag{D-56}$$

where $\tau_S = u_S\tau/2R$.

The values of $(DF)_T$ and $(DF)_A$ over a breathing cycle are calculated by superimposing DF for inspiration, deposition efficiency E during pause, and DF for expiration in the tracheobronchial airways and alveolar space. It is assumed that the breathing cycle consists of a constant flow inspiration, a pause, and a constant flow expiration, each with a respective duration fraction of 0.435, 0.05, and 0.515 of a breathing period.

## D.5.3. Lung Models

Lung architecture affects particle deposition in several ways: the linear dimension of the airway is related to the distance the particle travels before it contacts the airway surface; the air flow velocity by which the particles are transported is determined by the cross-section of the airway for a given volumetric flowrate; and flow characteristics in the airways are influenced by the airway diameter and branching patterns. Thus, theoretical prediction of particle deposition depends, to a large extent, on the lung model chosen.

**D.5.3.1. Lung Model for Rats** -- Morphometric data on the lung airways of rats were reported by Schum and Yeh (1979). Table D-1 shows the lung model data for Long Evans rats with a total lung capacity of 13.784cm$^3$. Application of this model to Fischer rats is accomplished by assuming that the rat has the same lung structure regardless of its strain and that the total lung capacity is proportional to the body weight. In addition, it is also assumed that the lung volume at rest is about 40% of the total lung capacity and that any linear dimension of the lung is proportional to the cubic root of the lung volume.

**D.5.3.2. Lung Model for Human Adults** -- The lung model of mature human adults used in the deposition calculation of DEPs is the symmetric lung model developed by Weibel (1963). In Weibel's model, the airways are assumed to be a dichotomous branching system with 24 generations. Beginning with the 18th generation, increasing numbers of alveoli are present on the wall of the airways and the last three generations are completely aleveolated. Thus, the alveolar region in this model consists of all the airways in the last seven

generations. Table D-2 presents the morphometric data of the airways of Weibel's model adjusted to a total lung volume of 3,000 $cm^3$.

**D.5.3.3. Lung Model for Children** -- The lung model for children in the diesel study was developed by Yu and Xu (1987) on the basis of available morphometric measurements. The model assumes a lung structure with dichotomous branching of airways, and it matches Weibel's model for a subject when evaluated at the age of 25 years, the age at which the lung is considered to be mature. The number and size of airways as functions of age t (years) are determined by the following equations:

**D.5.3.3.1. Number of airways and alveoli.** The number of airways $N_i(t)$ at generation i for age t is given by

$$N_i(t) = 2^i, \qquad \text{for } 0 \le i \le 20 \tag{D-57}$$

$$\begin{cases} N_{21}(t)=N_r(t), \\ N_{22}(t)=N_{23}(t)=0. \end{cases} \qquad \text{for } N_r(t) \le 2^{21} \tag{D-58}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = N_r(t) - 2^{21}, \\ N_{23}(t) = 0, \end{cases} \qquad \text{for } 2^{21} < N_r(t) \le 2^{22} \tag{D-59}$$

$$\begin{cases} N_{21}(t) = 2^{21}, \\ N_{22}(t) = 2^{22}, \\ N_{23}(t) = N_r(t) - 2^{21} - 2^{22} \end{cases} \qquad \textit{for } N_r(t) > 2^{21} + 2^{22}, \tag{D-60}$$

Table D-1 LUNG MODEL FOR RATS AT TOTAL LUNG CAPACITY

| Generation Number | Number of Airways | Length (cm) | Diameter (cm) | Accumulative Volume[b] (cm) |
|---|---|---|---|---|
| 1 | 1 | 2.680 | 0.340 | 0.243 |
| 2 | 2 | 0.715 | 0.290 | 0.338 |
| 3 | 3 | 0.400 | 0.263 | 0.403 |
| 4 | 5 | 0.176 | 0.203 | 0.431 |
| 5 | 8 | 0.208 | 0.163 | 0.466 |
| 6 | 14 | 0.117 | 0.134 | 0.489 |
| 7 | 23 | 0.114 | 0.123 | 0.520 |
| 8 | 38 | 0.130 | 0.112 | 0.569 |
| 9 | 65 | 0.099 | 0.095 | 0.615 |
| 10 | 109 | 0.091 | 0.087 | 0.674 |
| 11 | 184 | 0.096 | 0.078 | 0.758 |
| 12 | 309 | 0.073 | 0.070 | 0.845 |
| 13 | 521 | 0.075 | 0.058 | 0.948 |
| 14 | 877 | 0.060 | 0.049 | 1.047 |
| 15 | 1,477 | 0.055 | 0.036 | 1.414 |
| 16[a] | 2,487 | 0.035 | 0.020 | 1.185 |
| 17 | 4,974 | 0.029 | 0.017 | 1.254 |
| 18 | 9,948 | 0.025 | 0.016 | 1.375 |
| 19 | 19,896 | 0.022 | 0.015 | 1.595 |
| 21 | 39,792 | 0.020 | 0.014 | 2.003 |
| 22 | 79,584 | 0.019 | 0.014 | 2.607 |
| 25 | 318,336 | 0.017 | 0.014 | 7.554 |
| 24 | 636,672 | 0.017 | 0.014 | 13.784 |

[a]Terminal bronchioles
[b]Including the attached alveoli volume
(number of alveoli = $3 \times 10^7$, alveolar diameter = 0.0086 cm)

Table D-2 LUNG MODEL BY WEIBEL (1963) ADJUSTED TO 3,000 $CM^3$ LUNG VOLUME

| Generation Number | Number of Airways | Length (cm) | Diameter (cm) | Accumulative Volume[b] (cm) |
|---|---|---|---|---|
| 0 | 1 | 10.260 | 1.539 | 19.06 |
| 2 | 2 | 4.070 | 1.043 | 25.63 |
| 2 | 4 | 1.624 | 0.710 | 28.63 |
| 3 | 8 | 0.650 | 0.479 | 29.50 |
| 4 | 16 | 1.086 | 0.385 | 31.69 |
| 5 | 32 | 0.915 | 0.299 | 33.75 |
| 6 | 64 | 0.769 | 0.239 | 35.94 |
| 7 | 128 | 0.650 | 0.197 | 38.38 |
| 8 | 256 | 0.547 | 0.159 | 41.13 |
| 9 | 512 | 0.462 | 0.132 | 44.38 |
| 10 | 1,024 | 0.393 | 0.111 | 48.25 |
| 11 | 2,048 | 0.333 | 0.093 | 53.00 |
| 12 | 4,096 | 0.282 | 0.081 | 59.13 |
| 13 | 8,192 | 0.231 | 0.070 | 66.25 |
| 14 | 16,384 | 0.197 | 0.063 | 77.13 |
| 15 | 32,768 | 0.171 | 0.056 | 90.69 |
| 16[a] | 65,536 | 0.141 | 0.051 | 109.25 |
| 17 | 131,072 | 0.121 | 0.046 | 139.31 |
| 18 | 262,144 | 0.100 | 0.043 | 190.60 |
| 19 | 524,283 | 0.085 | 0.040 | 288.16 |
| 20 | 1,048,576 | 0.071 | 0.038 | 512.94 |
| 21 | 2,097,152 | 0.060 | 0.037 | 925.04 |
| 22 | 4,194,304 | 0.050 | 0.035 | 1,694.16 |
| 23 | 8,388,608 | 0.043 | 0.035 | 3,000.00 |

[a]Terminal bronchioles
[b]Including the attached alveolar volume
(number of alveoli = $3 \times 10^3$, alveolar diameter = 0.0288 cm)

where $N_r(t)$ is the total number of airways in the last three airway generations. The empirical equation for $N_r$ which best fits the available data is

$$N_r(t) = \begin{cases} 2.036 \times 10^7(1-0.926e^{-0.15t}), & t \leq 8 \\ 1.468 \times 10^7, & t > 8 \end{cases} \quad \text{(D-61)}$$

Thus, $N_r(t)$ increases from approximately 1.5 million at birth to 15 million at 8 years of age and remains nearly constant thereafter. Equations D-58 to D-60 also imply that in the last three generations, the airways in the subsequent generation begin to appear only when those in the preceding generation have completed development.

The number of alveoli as a function of age can be represented by the following equation according to the observed data:

$$N_A(t) = 2.985 \times 10^8(1 - 0.919e^{-0.45t}) \quad \text{(D-62)}$$

The number of alveoli distributed in the unciliated airways at the airway generation level is determined by assuming that alveolization of airways takes place sequentially in a proximal direction. For each generation, alveolization is considered to be complete when the number of alveoli in that generation reaches the number determined by Weibel's model.

**D.5.3.3.2. Airway size.** Four sets of data are used to determine airway size during postnatal growth: (a) total lung volume as a function of age; (b) airway size as given by Weibel's model; (c) the growth pattern of the bronchial airways; and (d) variation in alveolar size with age. From these data, it is found that the lung volume, LV(t) at age t, normalized to Weibel's model at 4,800 $cm^3$ for an adult (25 years old), follows the equation

$$LV(t) = 0.959 \times 10^5(1 - 0.998e^{-0.002t}) \ (cm^3). \quad \text{(D-63)}$$

The growth patterns of the bronchial airways are determined by the following equations

$$D_i(t) - D_{iw} = \alpha_i[H(t) - H(25)], \tag{D-64}$$

$$L_i(t) - L_{iw} = \beta_i[H(t) - H(25)], \tag{D-65}$$

where $D_i(t)$ and $L_i(t)$ are, respectively, the airway diameter and length at generation i and age t, $D_{iw}$ and $L_{iw}$ the corresponding values for Weibel's model, $\alpha_i$ and $\beta_i$ are coefficients given by

$$\alpha_i = 3.26 \ x \ 10^{-2}\exp[-1.183 \ (i+1)^{0.5}] \tag{D-66}$$

$$\beta_i = 1.05\times10^{-6} \exp \ [10.1] \ (i+1)^{-0.2}] \tag{D-67}$$

and H(t) is the body height, which varies with age t in the form

$$H(t) = 1.82 \ x \ 10^2(1 - 0.725e^{-0.14}t) \ (cm). \tag{D-68}$$

For the growth patterns of the airways in the alveolar region, it is assumed that

$$\frac{D_i}{D_{iw}} = \frac{L_i}{L_{iw}} = \frac{D_a}{D_{aw}} = f(t), \quad for \ 17 \le i \le 23 \tag{D-69}$$

where $D_a$ is the diameter of an alveolus at age t, $D_{aw}$ = 0.0288 cm is the alveolar diameter for adults in accordance with Weibel's model, and f(t) is a function determined from

$$f(t) = \sqrt[3]{\frac{\{LV(t) - \sum_{i=0}^{16} \frac{\pi}{4} D_i^2(t) \ L_i(t) N_i(t)\}}{\{\sum_{i=17}^{23} \frac{\pi}{4} D_{iw}^2 L_{iw} N_i(t) + \frac{5\pi}{36} D_{aw}^3 N_A(t)\}}} \tag{D-70}$$

## D.6. TRANSPORT RATES

The values of transport rates $\lambda_{XY}^{(i)}$ for rats have been derived from the experimental data of clearance for diesel soot (Chan et al., 1981; Strom et al., 1987, 1988) and for the particle associated organics (Sun et al., 1984; Bond et al., 1986; Yu et al. 1991). These values are used in the present model of lung burden calculation and are listed below:

$$\lambda_{HG}^{(i)} = 1.73 \quad (i = 1,2,3) \tag{D-71}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(i)} = 0.00018 \tag{D-72}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \tag{D-73}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \tag{D-74}$$

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{D-75}$$

$$\lambda_{AL}^{(1)} = 0.00068\ [1 - \exp(-0.046 m_A^{1.62})] \tag{D-76}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4}\lambda_{AB}^{(i)} \qquad (i = 2,3) \tag{D-77}$$

$$\lambda_{AT}^{(i)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) \quad (i = 1,2,3) \tag{D-78}$$

$$\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AT}^{(1)} + \lambda_{AB}^{(1)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00086 \quad \text{(D-79)}$$

$$\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_a^{1.62}) + 0.0161 \quad \text{(D-80)}$$

$$\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = 0.012 \exp(-0.11 m_A^{1.76}) + 0.00068 \exp(-0.046 m_A^{1.62}) + 15.7 \quad \text{(D-81)}$$

where $\lambda_{XY}^{(i)}$ is the unit of day$^{-1}$, and $m_A \cong m_A^{(1)}$ is the particle burden (in mg) in the alveolar compartment.

Experimental data on the deposition and clearance of DEPs in humans are not available. To estimate the lung burden of DEPs for human exposure, it is necessary to extrapolate the transport rates $\lambda_{XY}^{(i)}$ from rats to humans. For organics, we assume that the transport rates are the same for rats and humans. This assumption is based upon the observation of Schanker et al. (1986) that the lung clearance of inhaled lipophilic compounds appears to depend only on their lipid/water partition coefficients and is independent of species. In contrast, the transport rates of diesel soot in humans should be different from that of rats, since the alveolar clearance rate, $\lambda_A$, of insoluble particles at low lung burdens for human adults is approximately seven times that of rats (Bailey et al., 1982), as previously discussed in Chapter 4.

No data are available on the change of the alveolar clearance rate of insoluble particles in humans due to excessive lung burdens. It is seen from equation D-79 that $\lambda_A^{(1)}$ for rats can be written in the form

$$\lambda_A^{(1)} = a \exp(-b m_A^c) + d \quad \text{(D-82)}$$

where a, b, c, and d are constants. The right-hand side of equation D-82 consists of two terms, representing, respectively, macrophage-mediated mechanical clearance and clearance by dissolution. The first term depends upon the lung burden, whereas the second term does not. To extrapolate this relationship to humans, we assume that the dissolution clearance term was independent of species and that the mechanical clearance term for humans varies in the same

proportion as in rats under the same unit surface particulate dose. This assumption results in the following expression for $\lambda_A^{(1)}$ in humans

$$\lambda_A^{(1)} = \frac{a}{P} \exp(-b(m_A/S)^c) + d \qquad \text{(D-83)}$$

where P is a constant derived from the human/rat ratio of the alveolar clearance rate at low lung burdens, and S is the ratio of the pulmonary surface area between humans and rats. equation D-83 implies that rats and humans have equivalent amounts of biological response in the lung to the same specific surface dose of inhaled DEPs.

From the data of Bailey et al. (1982), we obtain a value of $\lambda_A^{(1)} = 0.00169$ day$^{-1}$ for humans at low lung burdens. This leads to P = 14.4. Also, we find S=148 from the data of the anatomical lung model of Schum and Yeh (1979) for rats and Weibel's model for human adults. For humans less than 25 years old, we assume the same value for P, but S is computed from the data of the lung model for young humans (Yu and Xu 1987). The value of S for different ages is shown in Table D-3.

The equations for other transport rates that have a lung-burden-dependent component are extrapolated from rats to humans in a similar manner. The following lists the values of $\lambda_{XY}^{(i)}$ (in day$^{-1}$) for humans used in the present model calculation:

$$\lambda_{HG}^{(1)} = 1.73 \ (i = 1,2,3) \qquad \text{(D-84)}$$

$$\lambda_{HB}^{(1)} = \lambda_{TB}^{(1)} = \lambda_{LB}^{(1)} = \lambda_{AB}^{(1)} = 0.00018 \qquad \text{(D-85)}$$

$$\lambda_{HB}^{(2)} = \lambda_{TB}^{(2)} = \lambda_{LB}^{(2)} = \lambda_{AB}^{(2)} = 0.0129 \qquad \text{(D-86)}$$

$$\lambda_{HB}^{(3)} = \lambda_{TB}^{(3)} = \lambda_{LB}^{(3)} = \lambda_{AB}^{(3)} = 12.55 \qquad \text{(D-87)}$$

TABLE D-4. RATIO OF PULMONARY SURFACE AREAS BETWEEN HUMANS AND RATS AS A FUNCTION OF HUMAN AGE

| Age (Year) | Surface Area |
|---|---|
| 0 | 4.99 |
| 1 | 17.3 |
| 2 | 27.6 |
| 3 | 36.7 |
| 4 | 44.7 |
| 5 | 51.9 |
| 6 | 58.5 |
| 7 | 64.6 |
| 8 | 70.4 |
| 9 | 76.0 |
| 10 | 81.4 |
| 11 | 86.6 |
| 12 | 91.6 |
| 13 | 96.4 |
| 14 | 101 |
| 15 | 106 |
| 16 | 110 |
| 17 | 115 |
| 18 | 119 |
| 19 | 123 |
| 20 | 128 |
| 21 | 132 |
| 22 | 136 |
| 23 | 140 |
| 24 | 144 |
| 25 | 148 |

$$\lambda_{TG}^{(i)} = 0.693 \qquad (i = 1,2,3) \tag{D-88}$$

$$\lambda_{AL}^{(1)} = 0.00068\ \{1 - 0.0694\ \exp[-0.046(m_A/S)^{1.62}]\} \tag{D-89}$$

$$\lambda_{AL}^{(i)} = \frac{1}{4}\ \lambda_{AB}^{(i)} \qquad (i = 2,\ 3) \tag{D-90}$$

$$\begin{gathered}\lambda_{AT}^{(i)} = 0.0694\ \{0.012\ \exp[-0.11(m_A/S)^{1.76}] + \\ 0.00068\ \exp[-0.046(m_A/S)^{1.76}]\}\ (i = 1,\ 2,\ 3)\end{gathered} \tag{D-91}$$

$$\begin{gathered}\lambda_A^{(1)} = \lambda_{AL}^{(1)} + \lambda_{AB}^{(1)} + \lambda_{AT}^{(1)} = \\ 0.0694\ \{0.012\ \exp[-0.11(m_A/S)^{1.76}]\} + 0.00086\end{gathered} \tag{D-92}$$

$$\begin{gathered}\lambda_A^{(2)} = \lambda_{AL}^{(2)} + \lambda_{AT}^{(2)} + \lambda_{AB}^{(2)} = \\ 0.0694\{0.012\ \exp[-0.11(m_A/A)^{1.76}] + \\ 0.00068\ \exp[-0.046(m_A/S)^{1.76}]\} + 0.016\end{gathered} \tag{D-93}$$

$$\begin{gathered}\lambda_A^{(3)} = \lambda_{AL}^{(3)} + \lambda_{AT}^{(3)} + \lambda_{AB}^{(3)} = \\ 0.0694\ \{0.012\ \exp[-0.11(m_A/S)^{1.76}] + \\ 0.00068\ \exp[-0.046(m_A/S)^{1.76}\} + 15.7\end{gathered} \tag{D-94}$$

## D.7. RESULTS

### D.7.1. Simulation of Rat Experiments

To test the accuracy of the model, simulation results are obtained on the retention of diesel soot in the rat lung and compare with the data of lung burden and lymph node burden obtained by Strom et al. (1988). A particle size of 0.19 μm MMAD and a standard geometric deviation, $\sigma_g$ of 2.3 (as used in Strom's experiment) are used in the calculation.

The respiratory parameters for rats are based on their weight and calculated using the following correlations of minute volume, respiratory frequency, and growth curve data.

$$\text{Minute Volume} = 0.9W \ (cm^3/min) \qquad \text{(D-95)}$$

$$\text{Respiratory Frequency} = 475W^{-0.3} \ (1/min) \qquad \text{(D-96)}$$

where W is the body weight (in grams) as determined from the equation

$$W = 5+537T/(100+T), \text{ for } T \geq 56 \text{ days} \qquad \text{(D-97)}$$

in which T is the age of the rat measured in days

Equation D-95 was obtained from the data of Mauderly (1986) for rats ranging in age from 3 months to 2 years old; equation D-96 was obtained from the data of Strom et al. (1988); and equation D-59 was determined from the best fit of the experimental deposition data. Figures D-3 and D-4 show the calculated lung burden of diesel soot ($m_A^{(1)} + m_f^{(1)}$) and lymph node burden, respectively, for the experiment by Strom et al. (1988) using animals exposed to DEPs at 6 $mg/m^3$ for 1,3,6 and 12 weeks; exposed in all cases was 7 days/week and 20 hours daily. The solid lines represent the calculated accumulation of particles during the continuous exposure phase and the dashed lines indicate calculated post exposure retention. The agreement between the calculated and the experimental data for both lung and lymph node burdens during and after the exposure periods was very good.

Comparison of the model calculation and the retention data of particle-associated BaP in rats obtained by Sun et al. (1984) is shown in Figure D-5. The calculated retention is shown by the solid line. The experiment of Sun et al. consisted of a 30 minute exposure to diesel particles coated with [$^3H$] benzo[a]pyrene ($[^3H]$ - $BaP$) at a concentration of 4-6 μg/m$^3$ of air and followed by a post exposure period of over 25 days. The fast and slow phase of ($[^3H]$ - $BaP$) clearance half-times were found to be 0.03 day and 18 days, respectively. These corrospond to $\lambda_{AO}^{2} = 0.0385$ day $^{-1}$ and $\lambda_{AO}^{(3)} = 23.1$ day$^{-1}$ in our model, where $\lambda_{AO}^{(i)}$ is the value of $\lambda_{XY}^{(i)}$ at $m_A \rightarrow 0$. Figure D-5 shows that the

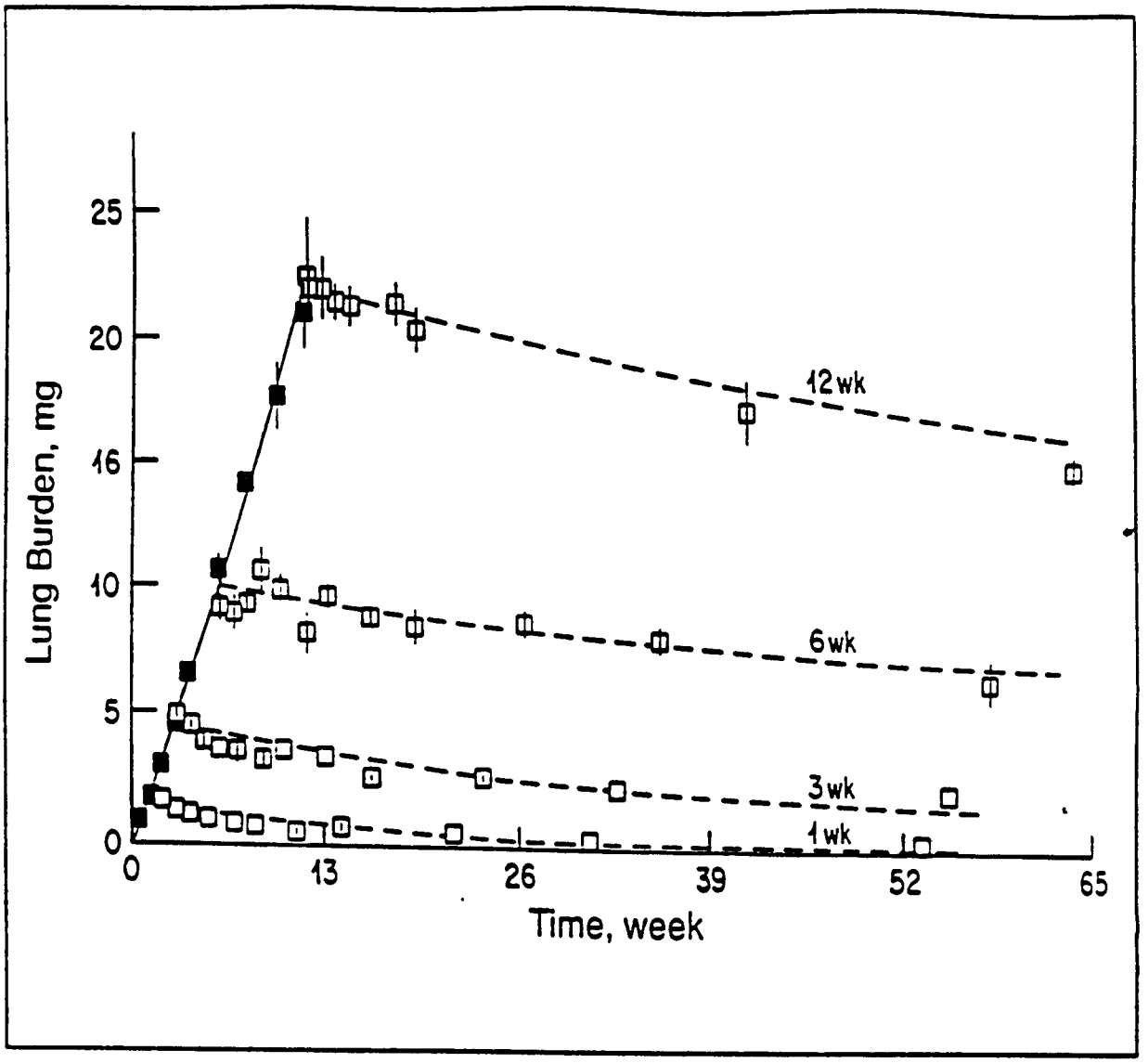


Figure D-3. The Experimental and predicted lung burdens of rats to DEPs at a solid and dashedconcentration of 0.6 mg/m$^3$ for different exposure spans.lines are, respectively, the predicted burdens during exposure and post exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

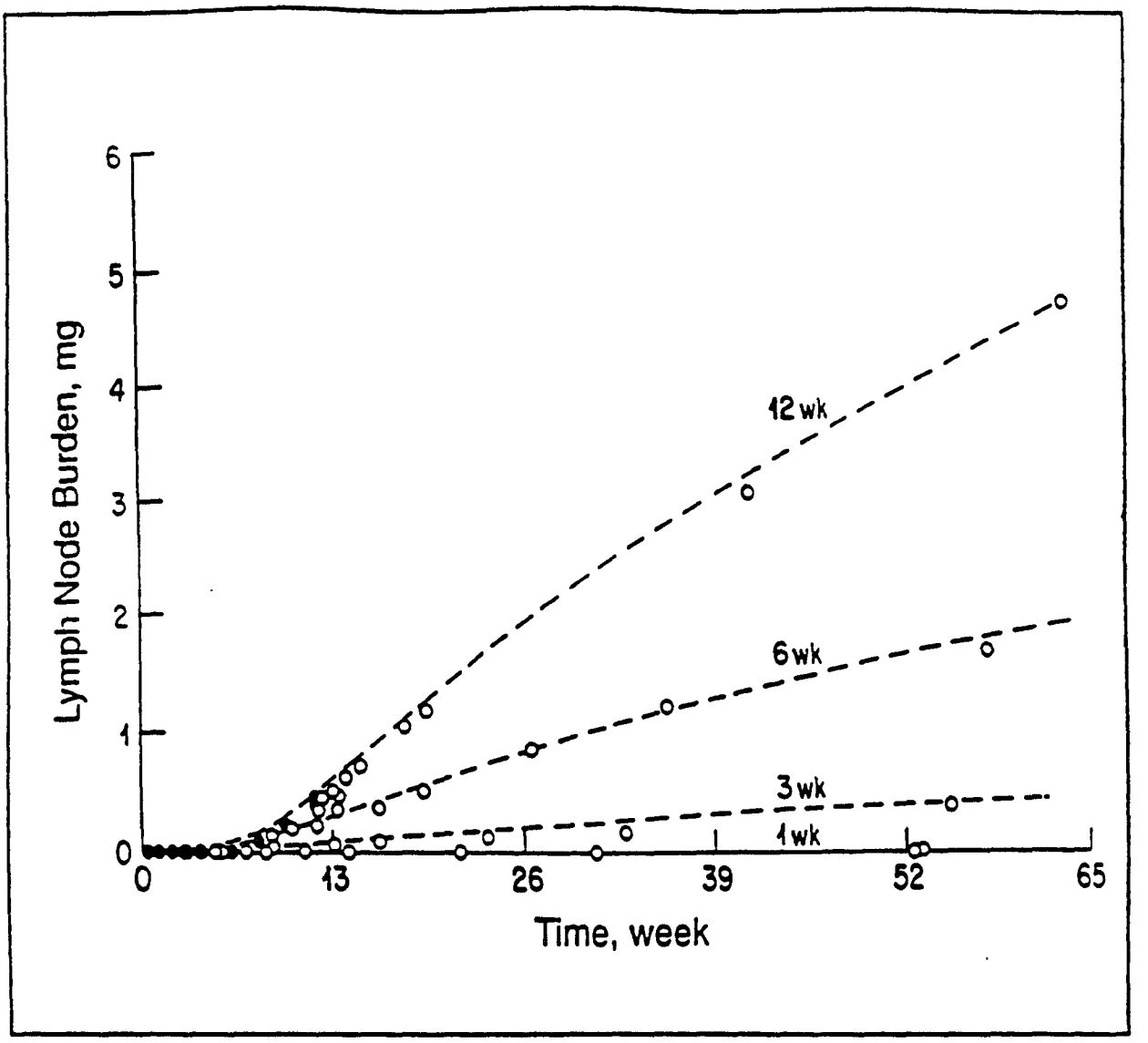


Figure D-4. Experimental and predicted lymph node burdens of rats exposed to DEPs at a concentration of 6.0 mg/m$^3$ for different exposure spans. The solid and dashed lines are, respectively, the predicted burdens during exposure and post exposure. Particle characteristics and exposure pattern are explained in the text. The symbols represent the experimental data from Strom et al. (1988).

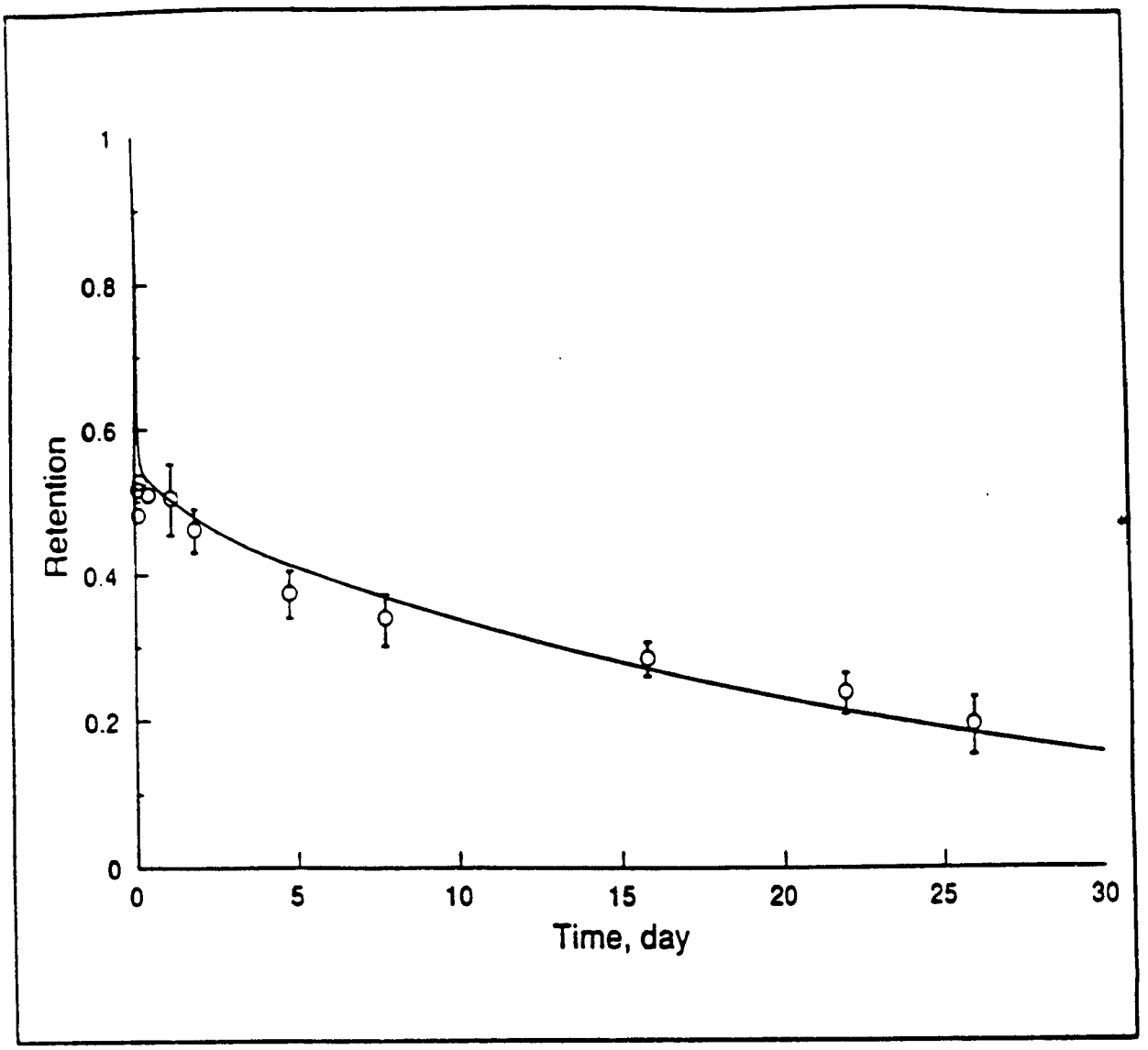


Figure D-5. Comparison between the calculated lung retention (solid line) and the experimental data obtained by Sun et al. (1984) for the particle associated BaP in rats.

calculated retention is in excellent agreement with the experimental data obtained by Sun et al., (1984).

### D.7.2. Predicted Burdens in Humans

Selected results of lung burden predictions in humans are shown in Figures D-6 to D-9. The particle conditions used in the calculation are 0.2 μm MMAD with $\sigma_g = 2.3$, and themass fractions of the rapidly and slowly cleared organics are each 10 percent $(f_1 = f_2 = 0.1)$. Figures D-6 and D-7 show, respectively, the lung burdens per unit concentration of diesel soot and the associated organics in human adults for different exposure patterns at two soot concentrations, 0.1 mg/m$^3$ and 1 mg/m$^3$. The exposure patterns used in the calculation are (a) 24 hours/day and 7 days week; (b) 12 hours/day and 7 days/week; and (c) 8 hours/day an 5days/week, simulating environmental and occupational exposure conditions. The results show that the lung burdens of both diesel soot and the associated organics reached a steady state value during exposure. Due to differences in the amount of particle intake, the steady state lung burdens per unit concentration were the highest for exposure pattern (a) and the lowest for exposure pattern (c). Also, increasing soot concentration from 0.1 mg/m$^3$ to 1 mg/m$^3$ increased the lung burden per unit concentration. However, the increase was not noticeable for exposure pattern (c). The dependence of lung burden on the soot concentration is caused by the reduction of the alveolar clearance rate at high lung burdens discussed above.

Figures D-8 and D-9 show the effect of age on lung burden, where the lung burdens per unit concentration per unit lung weight are plotted vs. age. The data of lung weight at different ages are those reported by Snyder (1975). The exposure pattern used in the calculation is 24 hours/day and 7 days/week for a period of one year at the two soot concentrations, 0.1 mg/m$^3$ and 1 mg/m$^3$. The results show that, on a unit lung weight basis, the lung burdens of both soot and the organics are functions of age and the maximum lung burdens ocurr at approxomately 5 years of age. Again, for any given age, the lung burden per unit concentration is slightly higher at 1 mg/m$^3$ than at 0.1 mg/m$^3$.

## D.8. PARAMETRIC STUDY OF THE MODEL

The deposition and clearance model of DEPs in humans, presented above, consists of a large number of parameters which characterize: the size and composition of diesel particles, the structure and dimension of the respiratory tract, the ventilation conditions of the subject,

and the clearance half-times of the diesel soot and the particle-associated organics. Any single or combined changes of these parameters from their normal values in the model would result in a change in the predicted lung burden. A parametric study has been conducted to investigate the effects of each individual parameter on calculated lung burden in human adults. The exposure pattern chosen for this study is 24 hours/day and 7 days/week for a period of 10 years at a constant soot concentration of 0.1 mg/m$^3$. The following presents two important results from the parametric study.

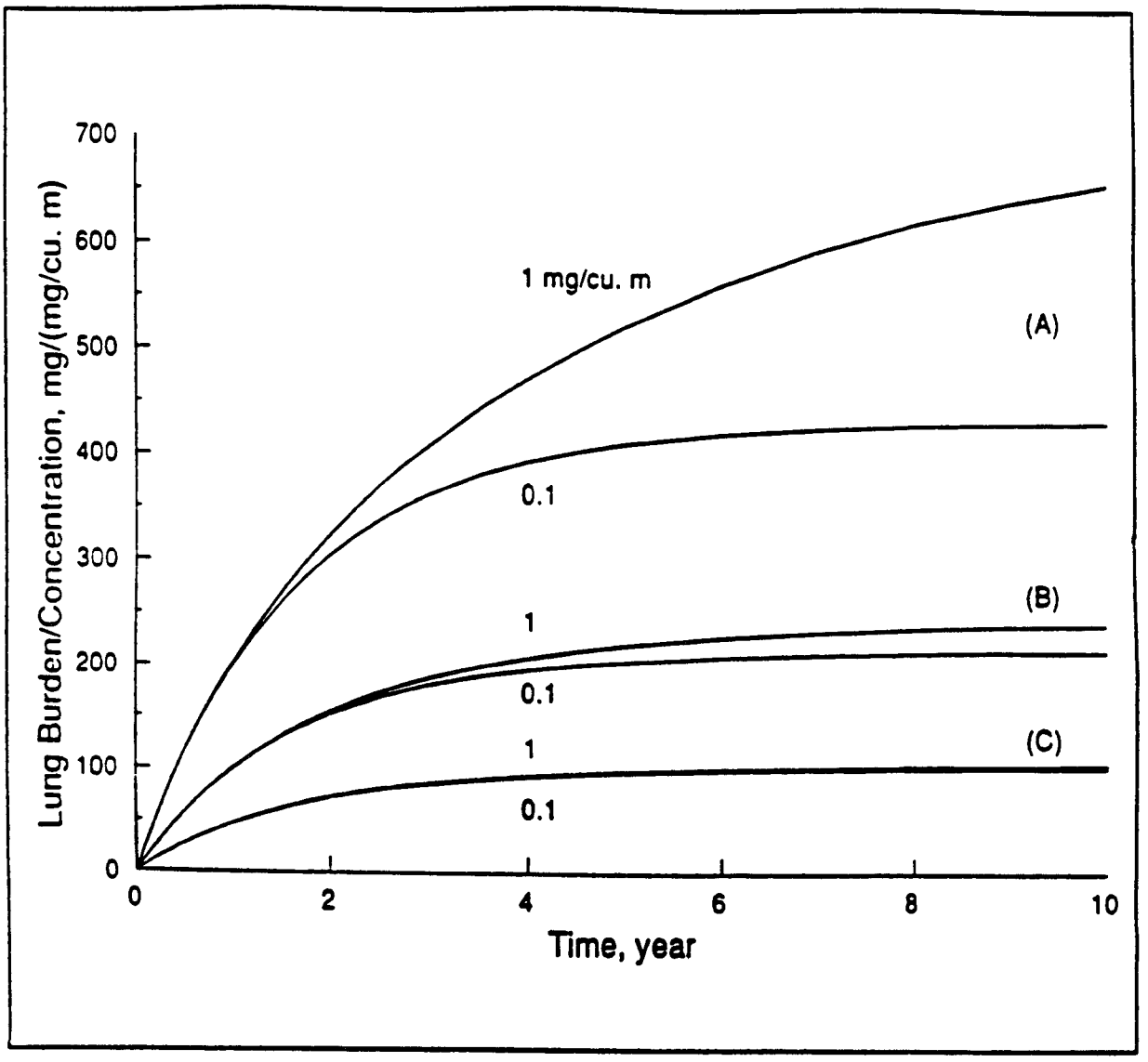


Figure D-6. Calculated lung burdens of diesel soot per unit exposure concentration in human adults exposed continueously to DEPs at two different concentrations of 0.1 mg/m$^3$ and 1.0 mg/m$^3$. Exposure patterns are (a) 24 hours/day and 7 days/week, (b) 12 hours/day and 7 days/week, and (c) 8 hours/day and 5 days/week.

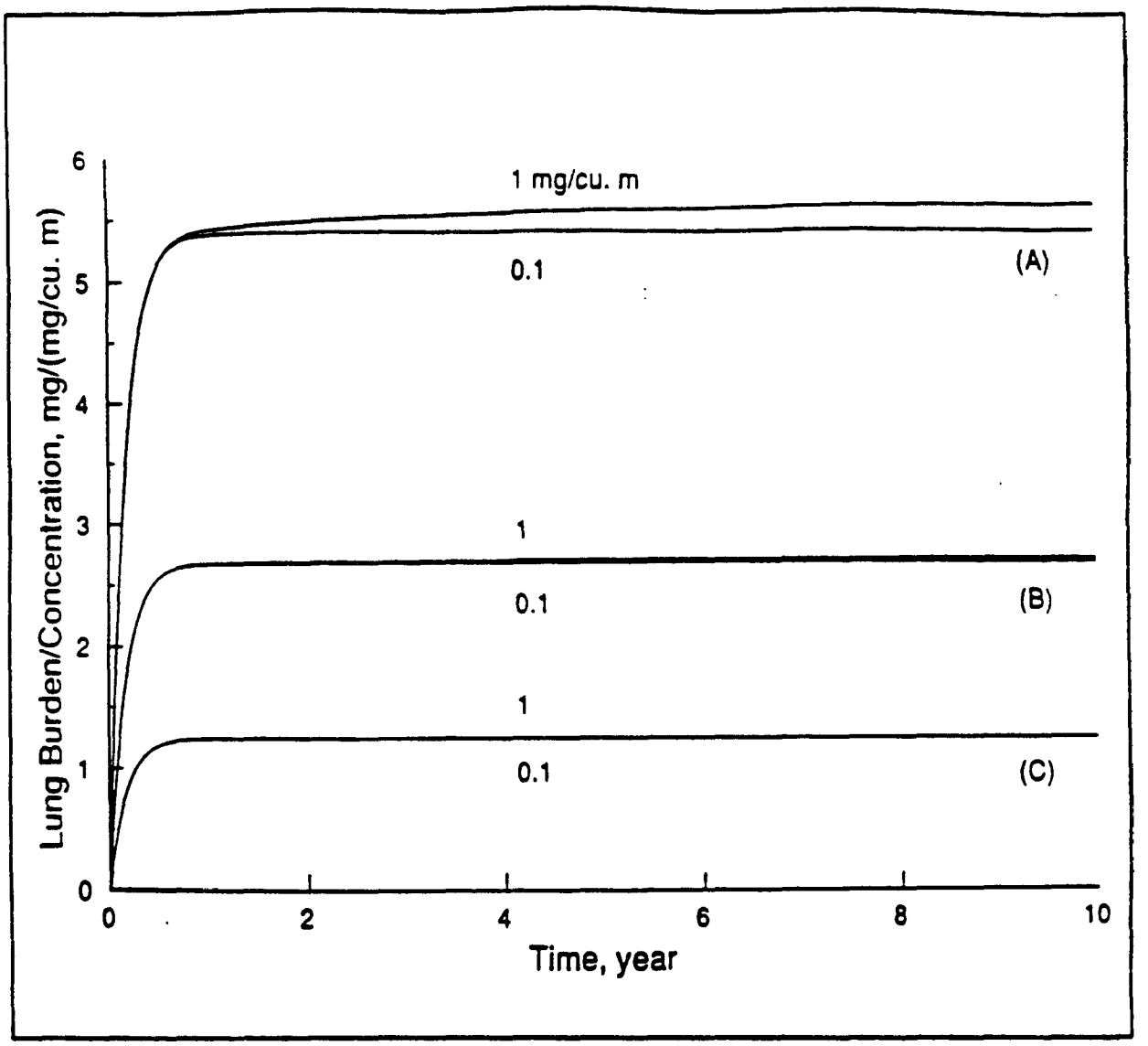


Figure D-7. Calculated lung burdens of the particle-associated organics per unit exposure concentration in human adults exposed continuously to DEPs at two different concentrations of 0.1 mg/m$^3$ and 1.0 mg/m$^3$. Exposure patterns are (a) 24 hours/day and 7 days/week, (b) 12 hours/day and 7 days/week, and (c) 8 hours/day and 5 days/week.

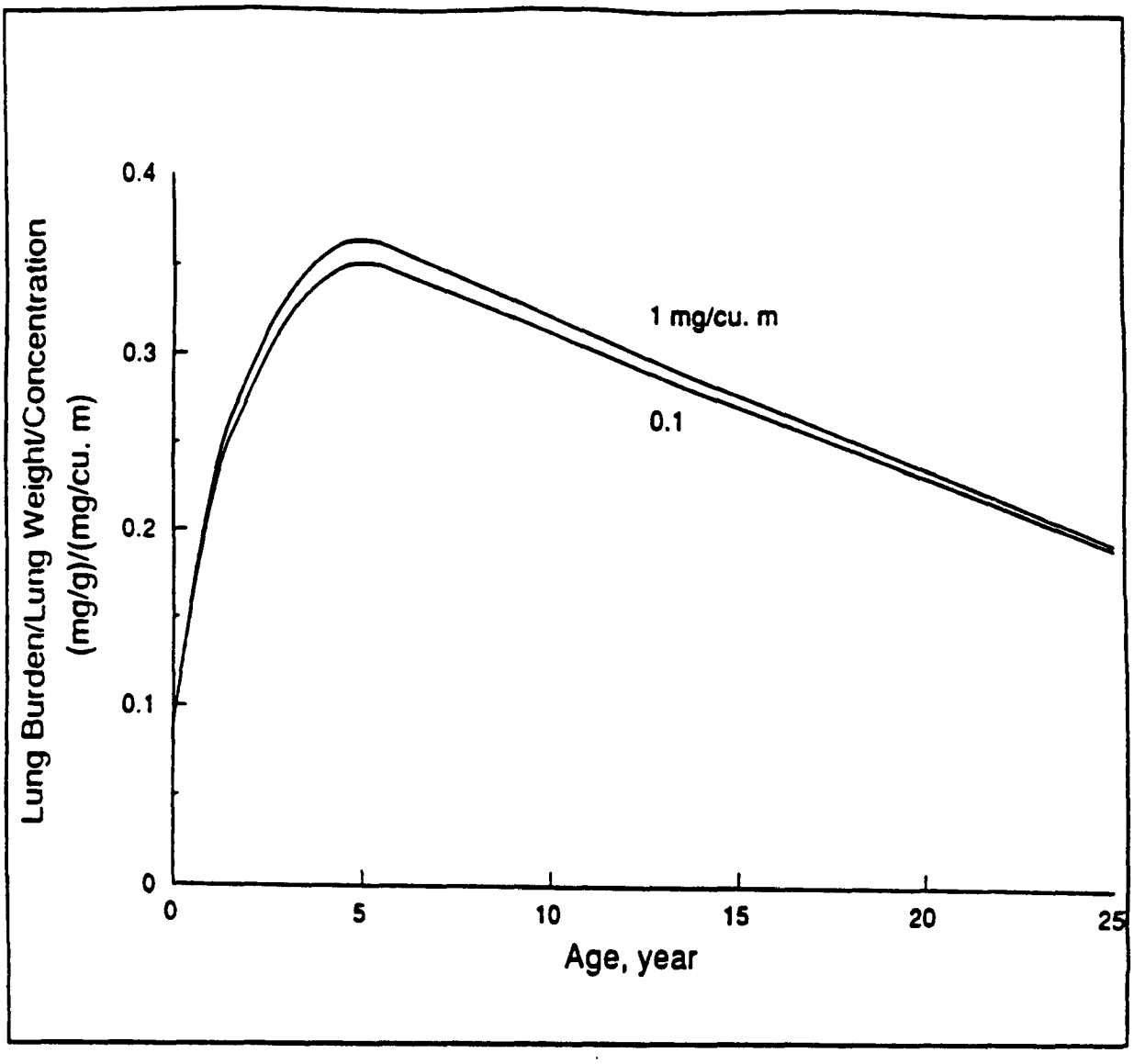


Figure D-8. Calculated lung burdens of diesel soot per gram of lung per unit exposure concentration in humans of different ages exposed continuously for one year to DEPs of two different concentrations of 0.1 mg/m$^3$ and 1.0 mg/m$^3$ for 7 days/week and 24 hours daily.

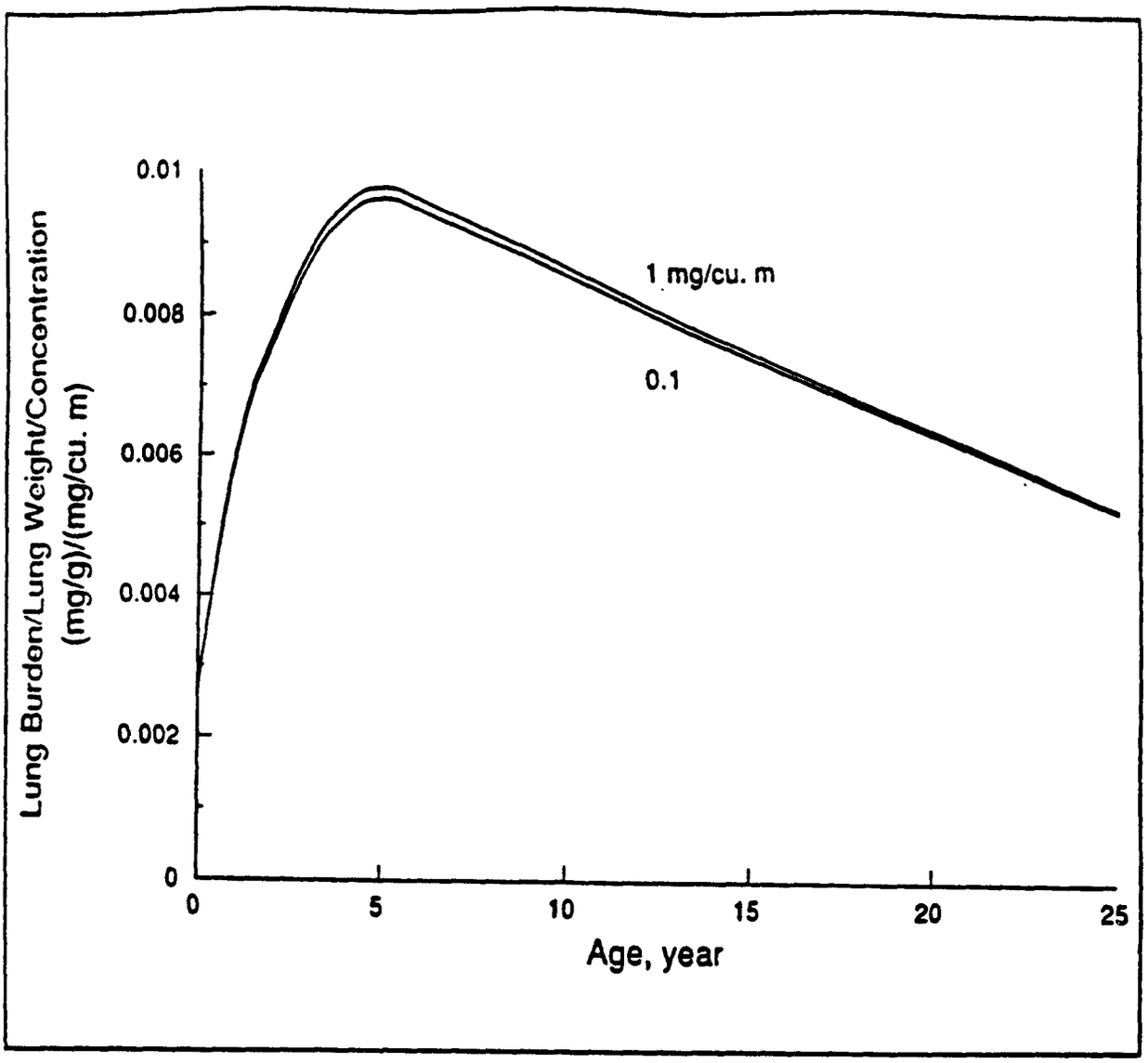


Figure D-9. Calculated burdens of the particle-associated organics per gram of lung per unit exposure concentration in humans of different ages exposed continuously for one year to DEPs of two different concentrations of 0.1 $mg/m^3$ and 1.0 $mg/m^3$ for 7 days/week and 24 hours daily.

### D.8.1. Effect of Ventilation Conditions

The change in lung vurden due to variations in tidal volume and respiratory frequency are depicted in Figures D-10 and D-11. Increasing any one of these ventilation parameters increased the lung burden, but the increase was much smaller with respect to respiratory frequency than to tidal volume. This small increase in lung burden was a result of the decrease in deposition efficiency as respiratory frequency increased, despite a higher total amount of DEPs inhaled.

The mode of breathing has only a minor effect on lung burden because switching from nose breathing does not produce any appreciable change in the amount of particle intake into the lung (Yu and Xu 1987). All lung burden results presented in this report are for nose breathing.

### D.8.2. Effect of Tranport Rates

Transport rates have an obvious effect on the retention of DEPs in the lung after depositon. Because we are mainly concerned with the long-term clearance of diesel soot and the associated organics, only the effects of two transport rates $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ are studied. Experimental data of $\lambda_A^{(1)}$ from various diesel studies in rats have shown that $\lambda_A^{(1)}$ can vary by a factor of two or higher. We use a multiple of 0.5 to 2 for the uncertainty in $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ to examine the effect on lung burden. Figures D-12 and D-13 show respectively, the lung burden results for diesel soot and the associated organics vs. the multiples of $\lambda_A^{(1)}$ and $\lambda_A^{(2)}$ used in the calculation. As expected, increasing the multiple of $\lambda_A^{(1)}$ reduced the lung burden of diesel soot with practically no change in the organics burden (Figure D-12), while just the oposite occurred when the multiple of $\lambda_A^{(2)}$ was increased (Figure D-13).

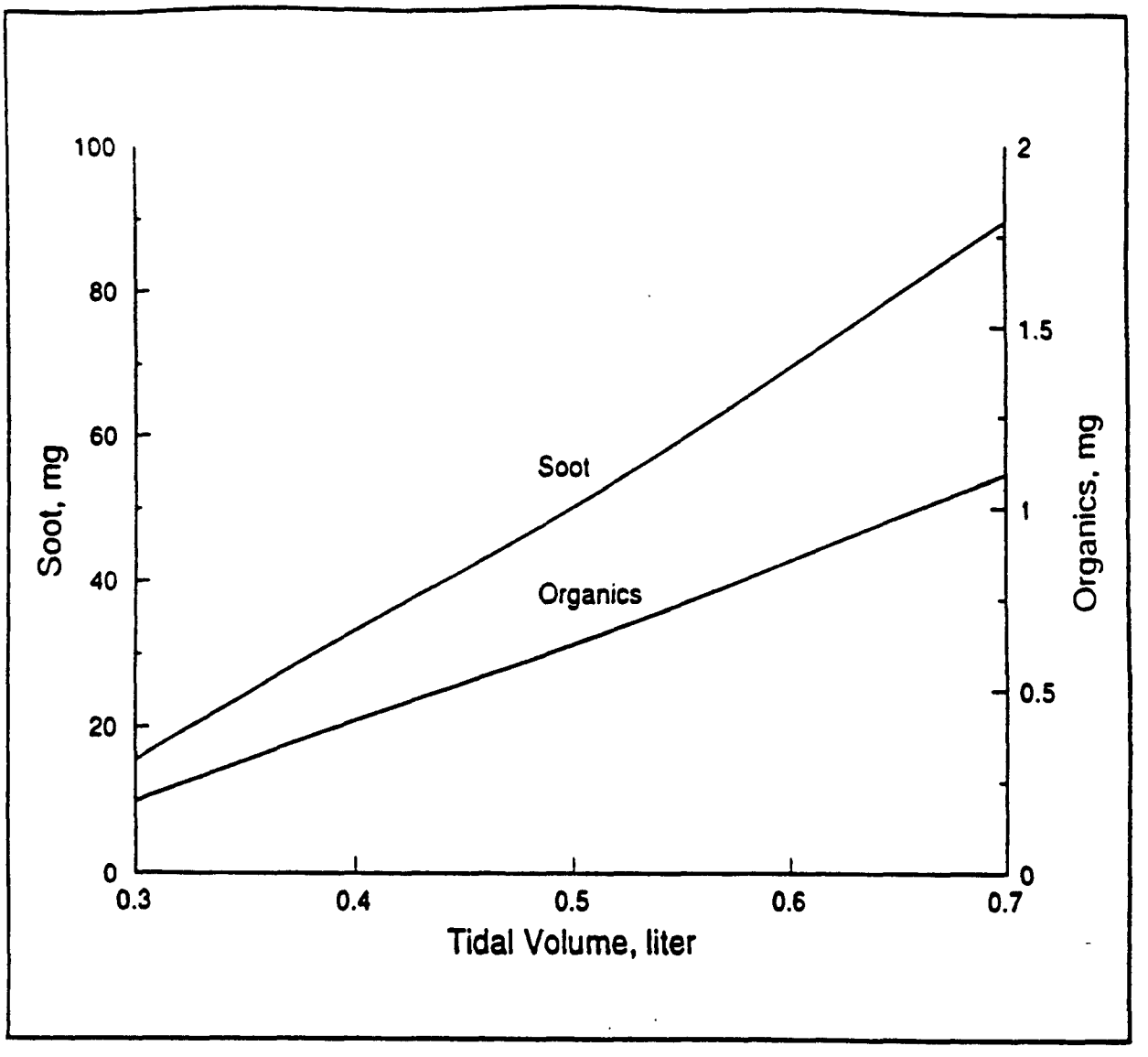


Figure D-10. Calculated lung burdens in human adults vs. tidal volume in liter for exposure to DEPs at 0.1 mg/m$^3$ for 10 years at 7 days/week and 24 hours daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, $\sigma_g$=2.3, $f_2$=0.1, $f_3$=0.1, (b) respiratory frequency = 14 min$^{-1}$, and (c) lung volume = 3000 cm$^3$.

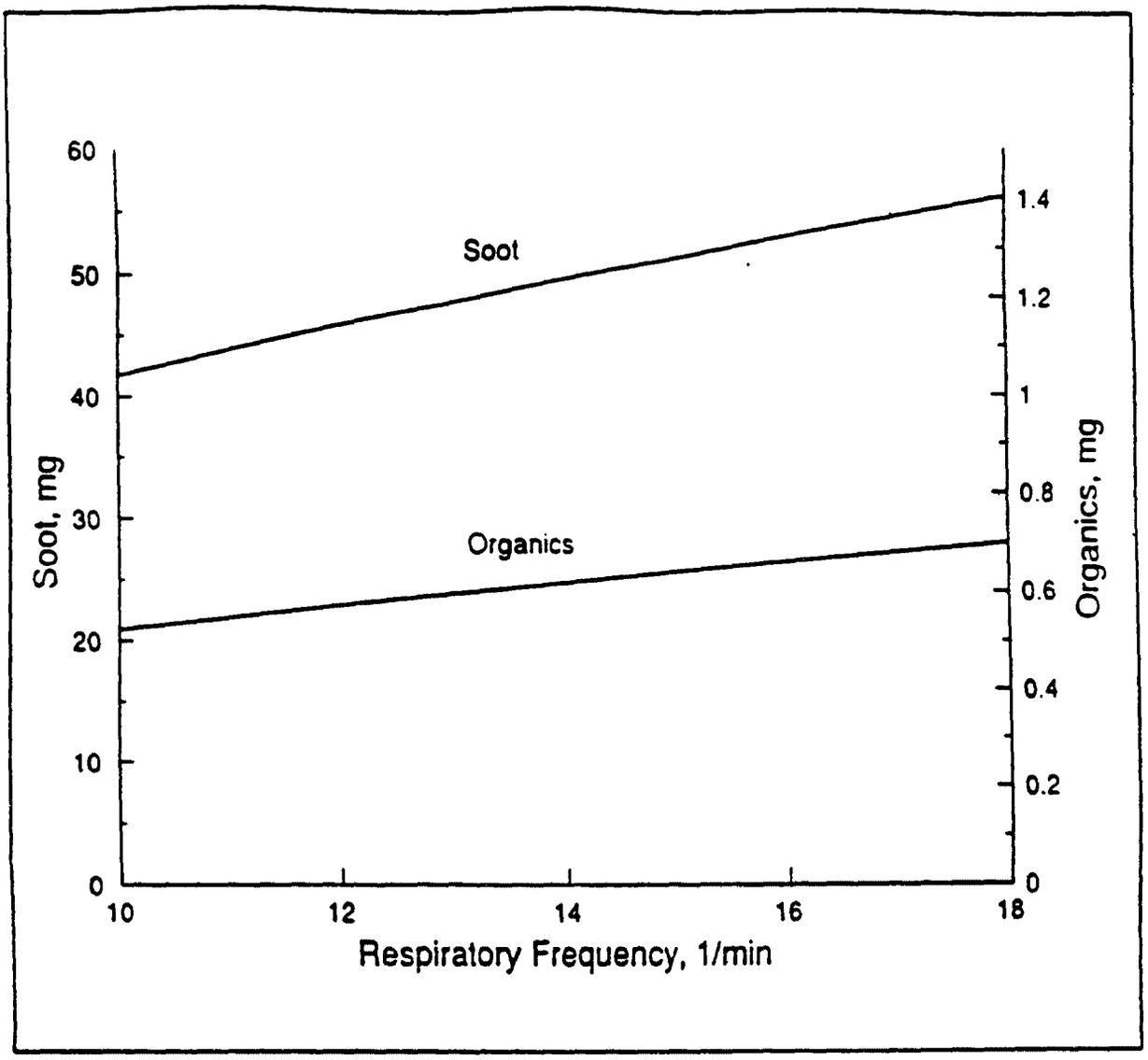


Figure D-11. Calculated lung burdens in human adults vs. respiratory freguency in *bpm* for exposure to DEPs at 0.1 mg/m$^3$ for 10 years at 7 days/week and 24 hours daily. Parameters used in the in the calculation are: (a) MMAD=0.2 μm, $\sigma_g$=2.3, $f_2$=0.1, $f_3$=0.1, (b) tidal volume = 500 cm$^3$, and (c) lung volume = 3200 cm$^3$.

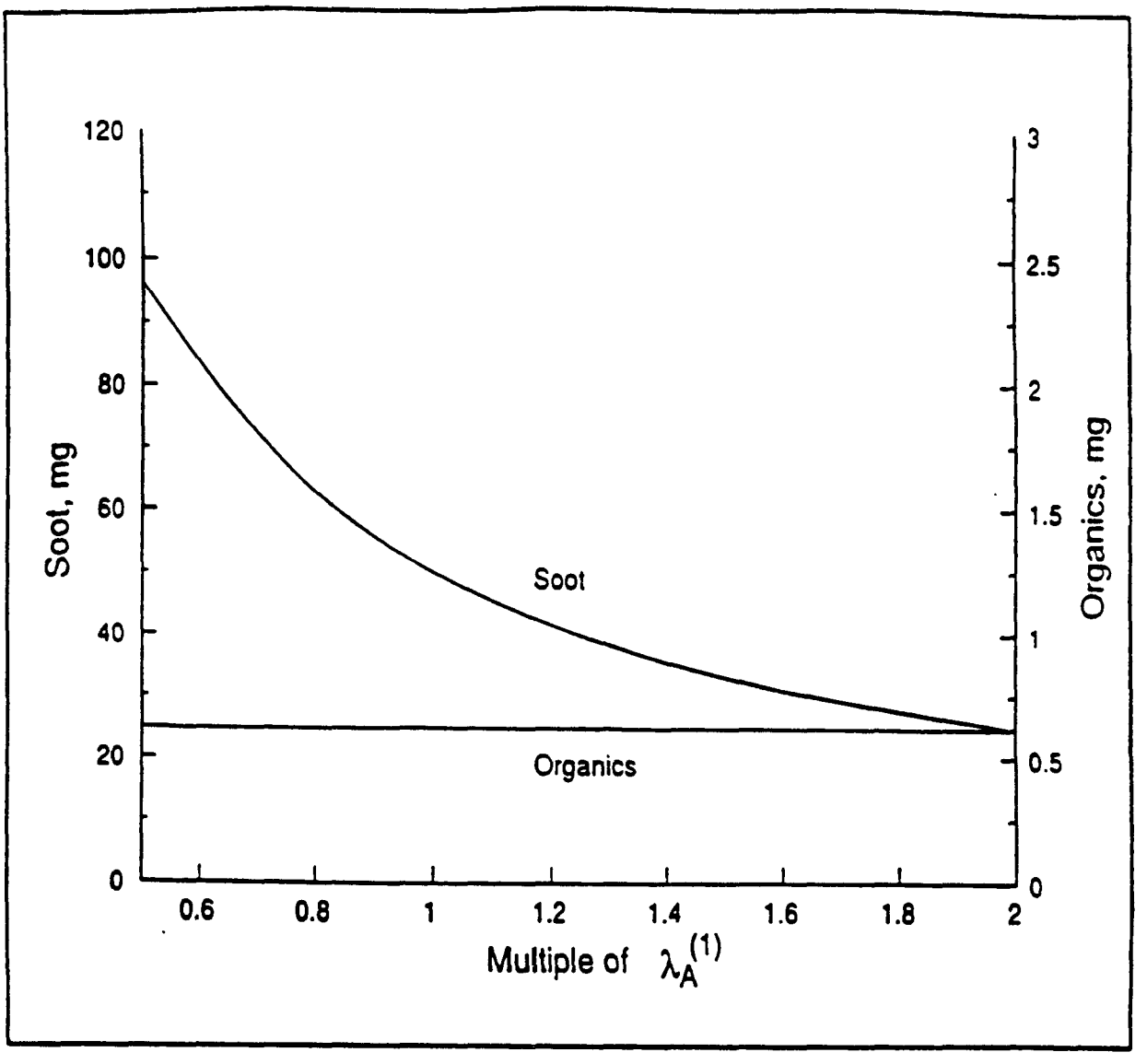


Figure D-12. Calculated lung burdens in human adults vs. multiple of $\lambda_A^{(1)}$ for exposure to DEPs at 0.1 mg/m$_3$ for 10 years at 7 days/week and 24 hours daily. Parameters used in the calculation are: (a) MMAD=0.2 μm, $\sigma_g$=2.3, $f_2$=0.1, $f_3$=0.1, (b) tidal volume = 500 cm$^3$, respiratory frequency = 14 min$^{-1}$, and (c) lung volume = 3200 cm$^3$.

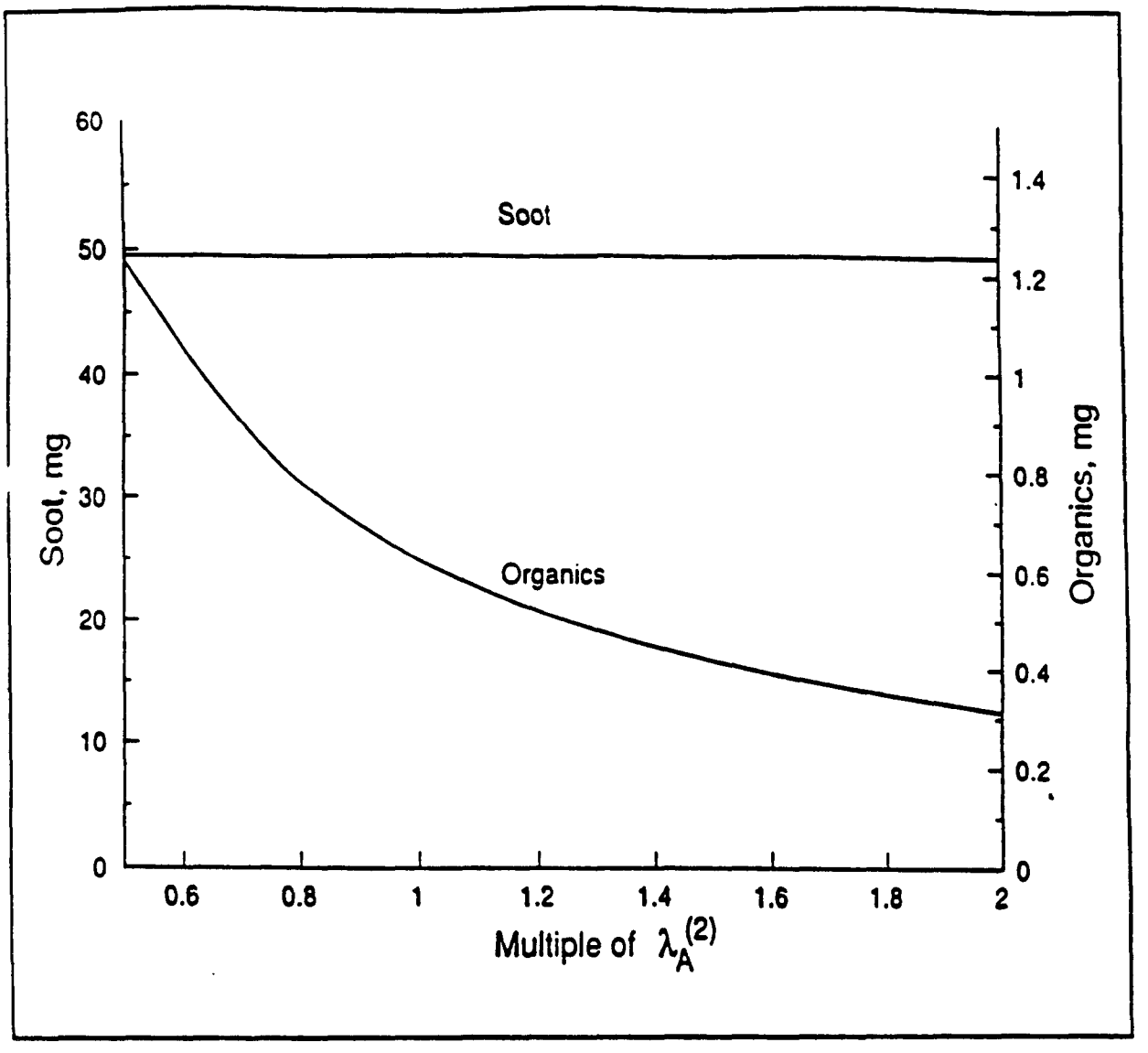


Figure D-13. Calculated lung burdens in human adults vs. multiple of $\lambda_A^{(1)}$ for exposure to DEPs at 0.1 mg/m$^3$ for 10 years at 7 days/week and 24 hours daily. Parameters used in the calculation are: (a) MMAD=0.2 μm $\sigma_g$=2.3, $f_2$=0.1, $f_3$=0.1, (b) tidal volume = 500 cm$^3$, respiratory frequency = 14 min$^{-1}$, and (c) lung volume = 3200 cm$^3$.

## D.9. REFERENCES


Amann, C.A.; Siegla, D.C. (1982) Diesel particles -- What are they and why. Aerosol Sci. Tech. 1:73-101

Bailey, M.R.; Fry, F.A.; James, A.C. (1982) The long-term clearance kinetics of insoluble particles from the human lung. Ann. Occup. Hyg. 26:273-289.

Bond, J.A.; Sun, J.D.; Medinsky, M.A.; Jones, R.K.; Yeh, H.C. (1986a) Deposition, metabolism and excretion of 1-[$^{14}$C]nitropyrene and 1-[$^{14}$C]nitropyrene coated on diesel exhaust particles as influenced by exposure concentration. Toxicol. Appl. Pharmacol. 85:102-117.

Chan, T.L.; Lee, P.S.; Hering, W.E. (1981) Deposition and clearance of inhaled diesel exhaust particles in the respiratory tract of Fisher rats. J. Appl. Tox. 1:77-82.

Diu, C.K.; Yu, C.P. (1983) Respiratory tract deposition of polydisperse aerosols in humans. Am. Ind. Hyg. Assoc. J. 44:62-65.

ICRP Publication 30, part 1. 1979. Limits for intakes of radionculides by workers. Ann ICRP 2.

Schanker, L.S.; Mitchell, E.W.; Brown, R.A. (1986) Species comparison of drug absorption from the lung after aerosol inhalation or intratracheal injection. Drug Metab. Dispos. 14(1):79-88.

Scheutzle, D. (1983) Sampling of vehicle emissions for chemical analysis and biological testing. Environ. Health Perspect. 47:65-80.

Schum, M.; Yeh, H.C. (1979) Theoretical evaluation of aerosol deposition in anatomical models of mammalian lung airways. Bull. Math. Biol. 42:1-15.

Snyder WS. 1975. Report of task group on reference man. pp. 151-173. Pergamon Press, Oxford, London.

Solderholm SC. 1981. Compartmental analysis of diesel particle kinetics in the respiratory sytem of exposed animals. Oral presentation at EPA Diesel Emissions Symposium, Raleigh, NC, October 5-7. In: Vostal JJ, Schreck RM, Lee PS, Chan TL, Soderholm SC. 1982. Deposition and clearance of diesel particles from the lung. In: Toxicological Effects of Emissions from Diesel Engins (Lewtas J, ed.) pp, 143-159. Elsevier, New York, NY.

Strom, K.A.; Chan, T.L.; Johnson, J.T. (1987) Pulmonary retention of inhaled submicron particles in rats: diesel exhaust exposures and lung retention model. Research Publication GMR-5718, Warren, MI: General Motors Research Laboratories.

Strom, K.A.; Chan, T.L.; Johnson, J.T. (1988) Inhaled particles VI. Dodgson, J.; McCallum, R.I.; Bailey, M.R.; Fischer, D.R., eds. London: Pergamon Press, pp. 645-658.

Sun, J.D.; Woff, R.K.; Kanapilly, G.M.; McClellan, R.O. (1984) Lung retention and metabolic fate of inhaled benzo(a)pyrene associated with diesel exhaust particles. Toxicol. Appl. Pharmacol. 73:48-59.

Weibel, E.R. (1963) Morphometry of the human lung. Berlin:Springer-Verlag.

Yu, C.P. (1978) Exact analysis of aerosol deposition during steady breathing. Powder Tech. 21:55-62.

Yu, C.P.; Diu, C.K.; Soong, T.T. (1981) Statistical analysis of aerosol deposition in nose and mouth. Am. Ind. Hyg. Assoc. J. 42:726-733.

Yu, C.P.; Xu, G.B. (1986) Predictive models for deposition of diesel exhaust particiates in human and rat lungs. Aerosol Sci. Tech. 5:337-347.

Yu, C.P.; Xu, G.B. (1987) Predicted deposition of diesel particles in young humans. J. Aerosol Sci. 18:419-429.

Yu, C.P., Yoon, K.J., Chen, Y.K. (1991) Retention modeling of diesel exhaust particles in rats and humans. J. Aerosol Medicine. In press.

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