of lymphocytes have been observed in the interstitium of lungs of cats exposed to diesel exhaust (6.3 mg/m$^3$ for 62 weeks, then 11.7 mg/m$^3$ for weeks 62–124; exposures were eight hours/day, seven days/week, for a total of 27 months) (Hyde et al. 1985). The subpopulations of these lymphocytes are not known, however. In lung lymphoid tissues, lymphoid cell numbers increased in rats and mice exposed to diesel exhaust (3.5 or 7.0 mg/m$^3$, seven hours/day, five days/week, for 6, 12, 18, and 24 months) (Bice et al. 1985). In guinea pigs exposed subacutely (1.5 mg/m$^3$, 20 hours/day, 5.5 days/week, for four or eight weeks), lymphoid cell numbers increased, but the subpopulation composition of B and T lymphocytes from tracheobronchial lymph nodes was unaltered by exhaust exposure (Dziedzic 1981). Increased numbers of lymphocytes also have been noted in the bronchoalveolar lavage fluid of rats exposed to diesel exhaust (1.5 or 0.75 mg/m$^3$, 20 hours/day, 5.5 days/week, for one year) (Strom 1984) and hamsters exposed to diesel exhaust (4.2 mg/m$^3$, 19 hours/day, five days/week, for two years) (Heinrich et al. 1986); however, Strom (1984) noted that these cells could have been extracted from enlarged epithelial lymphoid tissues by the lavage procedure, and thus the finding could be artifactual. When lung immune function was evaluated, the total number of antibody-forming cells increased in immunized rats chronically exposed to high levels of diesel exhaust (Bice et al. 1985).

There are no indications that systemic immunity is affected by diesel exhaust exposure. Cell counts of B and T cells in the blood and spleen were unchanged in guinea pigs subacutely exposed to diesel exhaust (Dziedzic 1981), and splenic B and T cell function was normal in rats chronically exposed (Mentnech et al. 1984; Lewis et al. 1986). In rats exposed to high levels of diesel exhaust (7.0 mg/m$^3$) for as long as a year, serum levels of specific immunoglobulins IgM, IgG, and IgA were similar to those in air-exposed control animals (Bice et al. 1985). When Takafuji and associates (1987) administered ovalbumin mixed with diesel exhaust particles intranasally, however, a greater systemic IgE response was noted than with ovalbumin alone. The investigators attributed adjuvant activity to the diesel particle.

In summary, even under conditions in which diesel particles were translocated to pulmonary lymphoid tissues, as noted by the increase in pigmentation of these tissues, local immune function was not compromised. Data supporting this conclusion are limited, however. The biological significance of the increase in the number of lymphocytes in bronchoalveolar lavage fluid, lung interstitium, and lymphoid tissues is unknown. No evidence supports an effect of diesel exhaust on systemic immunity.

## INFLAMMATION

In animals exposed to diesel exhaust, influxes of macrophages and aggregates of particle-laden cells have been observed in histologic preparations (Wiester et al. 1980; Karagianes et al. 1981; White and Garg 1981; Garg 1983; Hyde et al. 1985; Ishinishi et al. 1986; Iwai et al. 1986; Lewis et al. 1986; McClellan et al. 1986; Mauderly et al. 1987, 1989, 1994; Heinrich et al. 1989; Strom et al. 1990) (details of experimental protocols and findings are summarized in Table 3). After as little as two weeks of diesel exhaust exposure, increased numbers of alveolar macrophages have been noted in alveolar spaces (White and Garg 1981). Filled with particles, macrophages migrated toward the bronchoalveolar junction, where they tended to accumulate and form aggregates. Particle-filled macrophages also can be found in the interstitium and lymphoid tissues. Although increased numbers of macrophages were apparent in the lungs, the number of macrophages recovered by bronchoalveolar lavage in chronic exposure studies did not necessarily increase (Strom 1984; Heinrich et al. 1986; Henderson et al. 1988b; Mauderly et al. 1989, 1994) (see Table 3); differences in macrophage recovery may be related, in part, to differences in lavage techniques.

Increases in PMN numbers also occurred (Strom 1984; Heinrich et al. 1986; Mauderly et al. 1989, 1994) (see Table 3), and the cells appeared to follow the influx of macrophages into the alveoli (White and Garg 1981). It is presumed that macrophages release chemotactic factors that aid in the recruitment of the neutrophils. Eosinophils have been observed in studies that have used guinea pigs (Chen et al. 1980; Barnhart et al. 1981) and cats (Hyde et al. 1985).

The number of inflammatory cells remains elevated during exposure. Furthermore, Garg (1983) found that at least for six weeks after the cessation of exposure, macrophages continued to accumulate at the site of macrophage aggregates. The attachment of newly recruited macrophages to preexisting aggregates may also contribute to the variability in the number of cells recovered by bronchoalveolar lavage. Because these inflammatory cells are a source of mediators of inflammation, which can modulate the cellular sequelae to diesel exposure, more attention could be directed at measuring mediator levels and relating these levels to diesel exposure. Using acute exposure conditions (3.5 mg/m$^3$, seven hours/day, five days/week, for up to 17 days), Henderson and associates (1988a) measured increases in arachidonate metabolites in the bronchoalveolar lavage fluid from exposed rodents and compared levels of these metabolites in rats and mice. In chronic studies, increased levels of indicators of inflammation (e.g., cytoplasmic and lysosomal

**Table 3.** Effects of Diesel Exhaust Exposure on the Presence of Inflammatory Cells in the Lungs[a]

| Species | Exposure Conditions | Duration (months) | Particle Concentration ($mg/m^3$)[b] Effect[c] | | | | Reference |
|---|---|---|---|---|---|---|---|
| **Aggregates of Macrophages in Histologic Preparations** | | | | | | | |
| | | | **11.7 (Year 2)** | **6.3 (Year 1)** | | | |
| Cat | 8 hours/day, 7 days/week | 27 | ↑ | NA | | | Hyde et al. 1985 |
| | | | **8.3** | | | | |
| Rat | 6 hours/day, 5 days/week | 4 | + | | | | Karagianes et al. 1981 |
| | | 20 | + | | | | |
| | | | **6.5** | **2.5** | | | |
| Rat | 16 hours/day, 5 days/week | 3 | + | + | | | Mauderly et al. 1994 |
| | | 6 | + | + | | | |
| | | 12 | + | + | | | |
| | | 18 | + | + | | | |
| | | 23 | + | + | | | |
| | | | **6.3** | | | | |
| Guinea pig | 20 hours/day, 1 day/week | 2 | + | | | | Wiester et al. 1980 |
| | | | **6.0** | | | | |
| Rat | 20 hours/day, 5 days/week | 2 | + | | | | White and Garg 1981 |
| | | | **4.9** | | | | |
| Rat | 8 hours/day, 7 days/week | 1 | ± | | | | Iwai et al. 1986 |
| | | 3 | ± | | | | |
| | | 6 | + | | | | |
| | | | **4.0** | **2.0** | **1.0** | **0.4** | |
| Rat | 16 hours/day, 6 days/week, heavy-duty engine | 30 | + | + | + | – | Ishinishi et al. 1986 |
| | | | **2.0** | **1.0** | **0.4** | **0.1** | |
| | 16 hours/day, 6 days/week, light-duty engine | 30 | + | + | + | – | Ishinishi et al. 1986 |
| | | | **3.7** | | | | |
| Hamster | 19 hours/day, 5 days/week | 6 | ± | | | | Heinrich et al. 1989 |
| | | 10.5 | ± | | | | |
| | | 15 | + | | | | |
| | | 18 + clean air | + | | | | |

*(Table continues next page.)*

**Table 3.** Effects of Diesel Exhaust on the Presence of Inflammatory Cells in the Lungs[a] *(continued)*

| Species | Exposure Conditions | Duration (months) | Particle Concentration ($mg/m^3$)[b] Effect[c] | | | Reference |
|---|---|---|---|---|---|---|
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 12 | + | | | Mauderly et al. 1989 |
| | | 18 | + | | | |
| | | 24 | + | | | |
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 6 | + | | | Mauderly et al. 1987 |
| | | | **2.0** | | | |
| Rat | 7 hours/day, 5 days/week, heavy-duty engine | 3 | + | | | Lewis et al. 1986 |
| | | 6 | + | | | |
| | | 12 | + | | | |
| | | 24 | + | | | |
| | | | **0.5** | | | |
| Rat | 20 hours/day, 7 days/week | 1 | – | | | Strom et al. 1990 |
| | | 3.2 | + | | | |
| | | 6.5 | + | | | |
| | | 12 | + | | | |
| **Number of Macrophages in Bronchoalveolar Lavage Fluid** | | | | | | |
| | | | **7.0** | **3.5** | **0.35** | |
| Mice | 7 hours/day, 5 days/week | 6 | ↑ | NS | NS | Henderson et al. 1988b |
| | | 12 | ↑ | ↑ | NS | |
| | | 18 | ↑ | NS | NS | |
| Rat | 7 hours/day, 5 days/week | 6 | NS | NS | NS | Henderson et al. 1988b |
| | | 12 | NS | NS | NS | |
| | | 18 | NS | NS | NS | |
| | | 21 | ↑ | NS | NS | |
| | | | **6.5** | **2.5** | | |
| Rat | 16 hours/day, 5 days/week | 12 | NS | NS | | Mauderly et al. 1994 |
| | | 18 | ↑ | NS | | |
| | | 23 | NA | NS | | |
| | | | **4.2** | | | |
| Rat | 19 hours/day, 5 days/week | 12 | ↑ | | | Heinrich et al. 1986 |
| | | 24 | ↓ | | | |
| Hamster | 19 hours/day, 5 days/week | 12 | ↑ | | | Heinrich et al. 1986 |
| | | 24 | NS | | | |

*(Table continues next page.)*

**Table 3.** Effects of Diesel Exhaust on the Presence of Inflammatory Cells in the Lungs[a] *(continued)*

| Species | Exposure Conditions | Duration (months) | Particle Concentration ($mg/m^3$)[b] Effect[c] | | | Reference |
|---|---|---|---|---|---|---|
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 12 | NS | | | Mauderly et al. 1989 |
| | | 18 | ↓ | | | |
| | | 24 | ↓ | | | |
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 6 | NS | | | Mauderly et al. 1987 |
| | | | **1.5** | **0.75** | **0.25** | |
| Rat | 20 hours/day, 5.5 days/week | 6.5 | ↑ | ↑ | NS | Strom 1984 |
| | | 12 | ↑ | ↑ | NS | |
| **Number of Neutrophils in Bronchoalveolar Lavage Fluid** | | | | | | |
| | | | **7.0** | **3.5** | **0.35** | |
| Mice | 7 hours/day, 5 days/week | 6 | ↑ | ↑ | NS | Henderson et al. 1988b |
| | | 12 | ↑ | ↑ | NS | |
| | | 18 | ↑ | ↑ | NS | |
| Rat | 7 hours/day, 5 days/week | 6 | ↑ | NS | NS | Henderson et al. 1988b |
| | | 12 | ↑ | ↑ | NS | |
| | | 18 | ↑ | ↑ | NS | |
| | | 24 | ↑ | ↑ | NS | |
| | | | **6.5** | **2.5** | | |
| Rat | 16 hours/day, 5 days/week | 12 | ↑ | NS | | Mauderly et al. 1994 |
| | | 18 | ↑ | ↑ | | |
| | | 23 | NA | ↑ | | |
| | | | **4.2** | | | |
| Rat | 19 hours/day, 5 days/week | 24 | ↑ | | | Heinrich et al. 1986 |
| Hamster | 19 hours/day, 5 days/week | 24 | ↑ | | | Heinrich et al. 1986 |
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 12 | NS | | | Mauderly et al. 1989 |
| | | 18 | ↑ | | | |
| | | 24 | ↑ | | | |

*(Table continues next page.)*

**Table 3.** Effects of Diesel Exhaust on the Presence of Inflammatory Cells in the Lungs[a] *(continued)*

| Species | Exposure Conditions | Duration (months) | Particle Concentration ($mg/m^3$)[b] Effect[c] | | | Reference |
|---|---|---|---|---|---|---|
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 6 | ↑ | | | Mauderly et al. 1987 |
| | | | **1.5** | **0.75** | **0.25** | |
| Rat | 20 hours/day, 5.5 days/week | 6.5 | ↑ | NS | NS | Strom 1984 |
| | | 12 | ↑ | ↑ | NS | |

[a] Results are described relative to control animals.

[b] Particle concentrations are in boldface, and resulting effect is given beneath rule in regular type.

[c] + = Present; ± = minimal; – = not present; ↑ = increased, ↓ = decreased; NS = not significantly different from value in control animals; NA = information not available.

enzymes, total protein, collagen) were measured in the bronchoalveolar lavage fluid from exposed animals (Heinrich et al. 1986; Henderson et al. 1988b; Mauderly et al. 1994). A state of chronic inflammation may have implications for the biologic activity of diesel exhaust.

In summary, diesel exhaust exposure caused a marked inflammatory response. The influx of inflammatory cells (alveolar macrophages, PMNs, and eosinophils) and alveolar macrophage aggregates, and the increased levels of mediators, enzymes, and total protein in bronchoalveolar lavage fluid, have been measured in animals exposed to diesel exhaust.

## PROLIFERATION OF EPITHELIAL CELLS

Several investigators have described hyperplasia of bronchiolar and alveolar type II cells in the lungs of animals exposed to diesel exhaust (Wiester et al. 1980; Barnhart et al. 1981; White and Garg 1981; Hyde et al. 1985; Heinrich et al. 1986; Ishinishi et al. 1986; Iwai et al. 1986; Lewis et al. 1986; McClellan et al. 1986; Wright 1986; Mauderly et al. 1989, 1994) (details of experimental protocols and findings are summarized in Table 4). Hyperplasia has been reported in rats, mice, hamsters, guinea pigs, and cats. Hyperplastic foci usually begin as focal lesions, which may become more diffuse with continued exposure. Frequently, the foci are in close proximity to macrophage accumulations.

Few studies, however, have used assays that quantify cell turnover, such as labeling indices, DNA synthesis, or morphometry. Wright (1986) measured an increase in labeling indices of type II alveolar epithelial cells and in DNA synthesis in lung tissue from rats acutely exposed to diesel exhaust (6.0 $mg/m^3$, 20 hours/day, seven days/week, for up to 14 days). These responses peaked at two to three days of exposure, and the values returned to normal after seven days of exposure. Barnhart and colleagues (1981) measured an increase in the absolute volume of type II cells in guinea pigs exposed to diesel exhaust (0.75 $mg/m^3$ for two weeks or three months and 1.5 $mg/m^3$ for six months). Hyde and coworkers (1985) measured an increase in the labeling indices of the terminal bronchiolar epithelium in cats chronically exposed to diesel exhaust. In contrast to the description by Iwai and associates (1986), who reported that in rats the hyperplasia progressed after cessation of exposure, Hyde and coworkers (1985) did not find in cats a progression of the lesions after the exposed animals were allowed to recover in room air for six months. McClellan and coinvestigators (1986) measured the labeling index of epithelial cells in the large airways, terminal bronchioles, and alveoli of rats chronically exposed to diesel exhaust (7.0 $mg/m^3$, seven hours/day, five days/week) and correlated these measurements with the histopathology observed. Increased proliferation occurred in all three regions. In the pulmonary region, increases in labeling indices corresponded to histologically abnormal areas.

In summary, exposure to diesel exhaust caused hyperplasia of bronchiolar and alveolar type II cells. After cessation of exposure, progression of lesions was observed in rats, but not in cats. Quantitative measures of increased cellular proliferation correlated with hyperplastic foci observed histologically.

**Table 4.** Effects of Diesel Exhaust Exposure on Bronchiolar and Type II Epithelial Cell Proliferation[a]

| Species | Exposure Conditions | Duration | Particle Concentration ($mg/m^3$)[b] Effect[c] | | Reference |
|---|---|---|---|---|---|
| | | | **11.7 (Year 2)** | **6.3 (Year 1)** | |
| Cat | 8 hours/day, 7 days/week | 27 months | * | NA | Hyde et al. 1985 |
| | | | **7.0** | **0.35** | |
| Rat | 7 hours/day, 5 days/week | 18 months | + | NS | McClellan et al. 1986 |
| | | | **6.5** | **2.5** | |
| Rat | 16 hours/day, 5 days/week | 3 months | + | + | Mauderly et al. 1994 |
| | | 6 months | + | + | |
| | | 12 months | + | + | |
| | | 18 months | + | + | |
| | | 23 months | + | + | |
| | | | **6.3** | | |
| Guinea pig | 20 hours/day, 7 days/week | 2 months | + | | Wiester et al. 1980 |
| | | | **6.0** | | |
| Rat | 20 hours/day, 7 days/week | 1 day | NS | | Wright 1986 |
| | | 2 days | * | | |
| | | 3 days | * | | |
| | | 7 days | NS | | |
| | | 14 days | NS | | |
| | | | **6.0** | | |
| Rat | 20 hours/day, 5 days/week | 6 hours | NA | | White and Garg 1981 |
| | | 1 day | + | | |
| | | 3 days | NA | | |
| | | 1 week | NA | | |
| | | 2 weeks | NA | | |
| | | 4 weeks | NA | | |
| | | 6 weeks | + | | |
| | | 9 weeks | NA | | |
| | | | **4.9** | | |
| Rat | 8 hours/day, 7 days/week | 6 months | + | | Iwai et al. 1986 |
| | | 12 months | + | | |
| | | 24 months | + | | |
| | | | **4.2** | | |
| Rat | 19 hours/day, 5 days/week | 35 months | + | | Heinrich et al. 1986 |

*(Table continues next page.)*

**Table 4.** Effects of Diesel Exhaust Exposure on Bronchiolar and Type II Epithelial Cell Proliferation[a] *(continued)*

| Species | Exposure Conditions | Duration | Particle Concentration ($mg/m^3$)[b] / Effect[c] | | | | Reference |
|---|---|---|---|---|---|---|---|
| | | | **4.2** | | | | |
| Mice | 19 hours/day, 5 days/week | 30 months | + | | | | Heinrich et al. 1986 |
| Hamster | 19 hours/day, 5 days/week | 30 months | + | | | | Heinrich et al. 1986 |
| | | | **4.0** | **2.0** | **1.0** | **0.4** | |
| Rat | 16 hours/day, 6 days/week, heavy-duty engine | 30 months | ± | + | ± | ± | Ishinishi et al. 1986 |
| | | | **2.0** | **1.0** | **0.4** | **0.1** | |
| | 16 hours/day, 6 days/week, light-duty engine | 30 months | + | + | ± | ± | Ishinishi et al. 1986 |
| | | | **3.7** | | | | |
| Hamster | 19 hours/day, 5 days/week | 6 months | ± | | | | Heinrich et al. 1989 |
| | | 10.5 months | ± | | | | |
| | | 15 months | ± | | | | |
| | | 18 months + clean air | + | | | | |
| | | | **3.5** | | | | |
| Rat | 7 hours/day, 5 days/week | 12 months | ± | | | | Mauderly et al. 1989 |
| | | 18 months | + | | | | |
| | | 24 months | + | | | | |
| | | | **2.0** | | | | |
| Rat | 7 hours/day, 5 days/week, heavy-duty engine | 3 months | + | | | | Lewis et al. 1986 |
| | | 6 months | + | | | | |
| | | 12 months | + | | | | |
| | | 24 months | + | | | | |
| | | | **1.5** | **0.75** | | | |
| Guinea pig | 20 hours/day, 5.5 days/week | 2 weeks | NA | * | | | Barnhart et al. 1981 |
| | | 3 months | NS | * | | | |
| | | 6 months | * | NS | | | |

[a] Results are described relative to control animals.

[b] Particle concentrations are in boldface, and resulting effect is given beneath rule in regular type.

[c] + = Present; ± = minimal; * = significantly different from value in control animals; NS = not significantly different from value in control animals; NA = information not available.

## METAPLASIA

Metaplasia refers to the replacement of one cell type by a different cell type. With squamous metaplasia, the normal epithelium is replaced by stratified squamous epithelium. Bronchiolar-alveolar metaplasia occurs when the alveolar epithelium is replaced by cells normally found in the bronchioles, such as Clara or ciliated cells. Focal areas of epithelial metaplasia can be observed in animals exposed to diesel engine exhaust (Plopper et al. 1983; Hyde et al. 1985; Heinrich et al. 1986, 1989; Ishinishi et al. 1986; Iwai et al. 1986; Mauderly et al. 1986, 1994). Metaplastic foci occurred in the alveoli and terminal bronchioles and were usually associated with fibrotic foci (Mauderly et al. 1986) or aggregations of macrophages (Ishinishi et al. 1986; Iwai et al. 1986). In cats, Hyde and coworkers (1985) determined that the nonciliated bronchiolar cell population (100% of the cells in clean air controls) was partially replaced by ciliated cells (44%) and basal cells (9%) in animals exposed to diesel exhaust. The scanning electron photomicrographs by Plopper and associates (1983) clearly illustrate the alveolar-bronchiolarization that occurred in these cats.

In summary, squamous and bronchiolar-alveolar metaplasia occurred in the terminal bronchioles and alveoli of animals exposed to diesel exhaust.

## ALTERATIONS IN CONNECTIVE TISSUE

Several parameters of the connective tissue matrix are altered with chronic exposure to high levels of diesel exhaust. Histologic studies and biochemical assays reveal abnormalities in rats, mice, hamsters, guinea pigs, and cats. Although most of the studies include a description of these alterations in connective tissue, they were not designed specifically to explore the hypothesis that exposure to diesel exhaust causes these alterations. The functional significance of reported alterations in connective tissue parameters is not easily determined. Histologic lesions are usually focal in nature and do not uniformly compromise lung function (see Pulmonary Function section above).

### Evidence for Fibrosis

Fibrotic lesions have been reported in rats exposed to diesel exhaust (Karagianes et al. 1981; Mauderly et al. 1988, 1989, 1994), hamsters (Heinrich et al. 1986), mice (Heinrich et al. 1986), and cats (Hyde et al. 1985) (details of experimental protocols and findings are summarized in Table 5). Most of the reports are qualitative, but Hyde and coworkers (1985) provided quantitative evidence of interstitial fibrosis in cats. Significant increases in the number of fibroblasts, interstitial macrophages, and collagen fibers were measured in animals exposed to diesel exhaust; the peribronchiolar connective tissue space in the proximal acinar region increased 2.7-fold in thickness. Fibrotic changes persisted and possibly even progressed in animals that were allowed to recover in room air for six months. In rats, McClellan and coinvestigators (1986) measured an increase in the labeling index of alveolar interstitial cells located in sections of tissue that exhibited abnormal histology. In rats exposed to a diesel exhaust particle concentration of 3.5 $mg/m^3$ for 24 months, approximately 8% of the parenchyma showed evidence of fibrosis; in rats exposed to 7.0 $mg/m^3$, approximately 12% of the parenchyma was fibrotic.

Thickening of the alveolar wall septum also has been noted in numerous studies (Wiester et al. 1980; Barnhart et al. 1981; White and Garg 1981; Plopper et al. 1983; Heinrich et al. 1986, 1989; Ishinishi et al. 1986; Mauderly et al. 1988, 1989, 1994). Fine fibrillar material was present in the alveolar septa (White and Garg 1981; Ishinishi et al. 1986; Mauderly et al. 1988; Heinrich et al. 1989), but some of the reported increase in thickness of the septum also was due to the transition from squamous epithelial to cuboidal epithelial cells (Plopper et al. 1983; Mauderly et al. 1994) and to the increased cellularity of the alveolar interstitium (Barnhart et al. 1981; White and Garg 1981; McClellan et al. 1986).

In addition to the histopathologic evidence for increased parenchymal tissue, results from biochemical studies show increased amounts of lung collagen (Hyde et al. 1985; Henderson et al. 1988b; Mauderly et al. 1989). In cats (Hyde et al. 1985) and rats (Misiorwski et al. 1980) exposed to diesel exhaust, increases in collagen content were attributed to newly synthesized protein. Increases in the collagen (Heinrich et al. 1986) and hydroxyproline (Henderson et al. 1988b) content in the bronchoalveolar lavage fluid of exposed rats and mice have also been reported.

### Mechanisms of Fibrotic Response

Pulmonary fibrosis appears to be a generic response of rats when they are chronically exposed to high levels of insoluble, "inert" particles resulting in lung overload. At predictable doses of retained insoluble particles, fibrosis developed in rats, even after exposure was terminated (reviewed by Mermelstein et al. 1992). The appearance of pulmonary fibrosis at specific lung doses has been noted for different particle types including toners, titanium dioxide, carbon black, and diesel exhaust.

Although potency among various fibrogenic agents differs, the development of fibrotic lesions from diesel exhaust exposure probably does not differ mechanistically from the development of fibrotic disease brought about by other fibrogenic agents. Inflammatory cells (macrophages, neutrophils, lymphocytes, and in some species, eosinophils)

come into the lung during diesel-particle exposure, with subsequent increases in fibroblasts and connective tissue. Cell-derived oxidants and myeloperoxidase have not been measured during the early phases of diesel exhaust exposure. Similarly, effector molecules have not been measured during "repair" of damaged tissues. Although the presence of these mediators of injury and repair can only be inferred, it is unlikely that diesel exhaust is different from other fibrogenic particulate agents. Histologic data from animal studies with coal dust and diesel exhaust suggest few differences in response to the two particulate agents. Exposures to either high (8.3 mg/m$^3$) (Karagianes et al. 1981) or low (2 mg/m$^3$) (Lewis et al. 1986) concentrations of diesel particles or coal dust produce similar histologic responses

**Table 5.** Effects of Diesel Exhaust Exposure on the Occurrence of Fibrotic Lesions[a]

| Species | Exposure Conditions | Duration (months) | Particle Concentration (mg/m$^3$)[b] Effect[c] | | | Reference |
|---|---|---|---|---|---|---|
| | | | **11.7 (Year 2)** | **6.3 (Year 1)** | | |
| Cat | 8 hours/day, 7 days/week | 27 | + | NA | | Hyde et al. 1985 |
| | | | **8.3** | | | |
| Rat | 6 hours/day, 5 days/week | 4 | ± | | | Karagianes et al. 1981 |
| | | 20 | + | | | |
| | | | **7.0** | **3.5** | **0.35** | |
| Rat | 7 hours/day, 5 days/week | 24 | + | + | – | Mauderly et al. 1988<br>McClellan et al. 1986 |
| | | | **6.5** | **2.5** | | |
| Rat | 16 hours/day, 5 days/week | 3 | – | – | | Mauderly et al. 1994 |
| | | 6 | ± | – | | |
| | | 12 | + | ± | | |
| | | 18 | + | + | | |
| | | 23 | + | + | | |
| | | | **4.2** | | | |
| Mice | 19 hours/day, 5 days/week | 24 | + | | | Heinrich et al. 1986 |
| | | | **3.7** | | | |
| Hamster | 19 hours/day, 5 days/week | 6 | –/+ | | | Heinrich et al. 1989 |
| | | 15 | ± | | | |
| | | 18 + clean air | ± | | | |
| | | | **3.5** | | | |
| Rat | 7 hours/day, 5 days/week | 24 | + | | | Mauderly et al. 1989 |

[a] Results are described relative to control animals.

[b] Particle concentrations are in boldface, and resulting effect is given beneath rule in regular type.

[c] + = Present; ± = minimal; – = not present; –/+ = rare.

in the lungs of rats. In addition, exposures of rats to diesel exhaust or carbon black particles using identical exposure protocols (2.5 or 6.5 mg/m$^3$, 16 hours/day, five days/week, for up to 24 months) resulted in similar histopathology.

### Evidence for Emphysema

In addition to lesions that reflect a stiffening of lung tissue, some studies report changes that suggest a regional loss of tissue mass (Karagianes et al. 1981; Heinrich et al. 1986; Mauderly et al. 1988, 1989) (see Table 6). Karagianes and colleagues (1981) described lesions resembling centrilobular emphysema, which progressed to panacinar emphysema with continued exposure. In animals exposed to a diesel exhaust particle concentration of 7 mg/m$^3$, Mauderly and coworkers (1988) measured increases in the mean linear intercept, which paralleled decreases in the internal surface area of the lungs. Often, but not always, emphysematous lesions were adjacent to areas of fibrosis. At a lower diesel particle concentration of 3.5 mg/m$^3$, focal lesions were apparent, but morphometric measurements did not show significant differences between control animals and those exposed to diesel exhaust (Mauderly et al. 1988, 1989). Similarly, Lewis and coinvestigators (1986), who also measured volume density, surface density, and mean linear intercept of the alveoli in rats exposed to exhaust particle concentrations of 2 mg/m$^3$ from a heavy-duty engine, did not find changes that were indicative of emphysema. Johnson and coinvestigators (1990) measured a reduction in the functional activity of alpha-1-proteinase inhibitor in rats chronically exposed to diesel exhaust.

In summary, exposure to diesel exhaust caused alveolar septal and focal interstitial fibrosis. Biochemical studies support histologic findings of lung fibrosis. Although not as pronounced as fibrosis, emphysematous lesions also occurred in animals exposed to diesel exhaust.

## MORTALITY

Although chronic exposure to diesel engine exhaust caused numerous effects in laboratory animals, the exhaust had little effect on mortality in rats and hamsters (Karagianes et al. 1981; Heinrich et al. 1986, 1989; Ishinishi et al. 1986; Mauderly et

**Table 6.** Effects of Diesel Exhaust Exposure on the Occurrence of Emphysematous Lesions[a]

| Species | Exposure Conditions | Duration (months) | Particle Concentration (mg/m$^3$)[b] / Effect[c] | | | Reference |
|---|---|---|---|---|---|---|
| Rat | 6 hours/day, 5 days/week | 4<br>20 | **8.3**<br>±<br>+ | | | Karagianes et al. 1981 |
| Rat | 7 hours/day, 5 days/week | 24 | **7.0**<br>+ | **3.5**<br>+ | **0.35**<br>– | Mauderly et al. 1988 |
| Hamster | 19 hours/day, 5 days/week | 24 | **4.2**<br>+ | | | Heinrich et al. 1986 |
| Rat | 7 hours/day, 5 days/week | 24 | **3.5**<br>+ | | | Mauderly et al. 1989 |
| Rat | 7 hours/day, 5 days/week, heavy-duty engine | 24 | **2.0**<br>– | | | Lewis et al. 1986 |

[a] Results are described relative to control animals.

[b] Particle concentrations are in boldface, and resulting effect is given beneath rule in regular type.

[c] + = Present, ± = minimal; – = not present.

al. 1986, 1989). Heinrich and coworkers (1986), however, reported an increase in mortality in mice exposed to 4.2 $mg/m^3$, 19 hours/day, five days/week, for two years. Also, female rats exposed to 6.5 $mg/m^3$, 16 hours/day, five days/week, for up to 23 months exhibited a shorter median life span than control female animals (Mauderly et al. 1994).

## SUMMARY

Exposure of animals to diesel engine exhaust caused an influx of inflammatory cells, both macrophages and PMNs, into the lungs. Hyperplasia, metaplasia, alterations in connective tissue, and pulmonary fibrosis have been reported to be associated with chronic inflammation. Although the evidence is incomplete, and in some cases contradictory, some evidence indicates that nonspecific host defenses may have been compromised with diesel exhaust exposure. The impact of diesel exhaust exposure on macrophage function was poorly characterized. Immune function, both pulmonary and systemic, appeared unaffected by diesel exhaust exposure. Finally, chronic exposure to diesel exhaust compromised pulmonary function. Impairments of function followed both restrictive and obstructive patterns of disease. The presence of these effects did not dramatically compromise the survival time of chronically exposed animals.

The significance of these reported effects from diesel exhaust exposure are twofold:

- Some of the observed effects, in particular chronic inflammation and epithelial cell proliferation, may be related to nongenotoxic mechanisms of carcinogenesis.
- Some of the effects may have an impact on health unrelated to tumor formation; for example, fibrosis occurred in rats chronically exposed to diesel exhaust. The implications for fibrotic disease in humans exposed to diesel exhaust are not known.

# EVALUATING DOSE RESPONSE

Recently, regulatory agencies and health organizations have been analyzing noncancer endpoints to establish threshold concentrations for exposures to diesel exhaust that do not cause any appreciable deleterious effects. Such analyses usually consist of two steps: (1) construction of a dose-response curve using experimental data and (2) extrapolation from animal models to human exposures using dosimetry models. More specifically, the U.S. Environmental Protection Agency (EPA) established an inhalation reference concentration (RfC) of 5 $\mu g/m^3$ for continuous lifetime exposure to diesel particulate matter (U.S. Environmental Protection Agency 1993). Because the data from human studies were considered inadequate, the EPA calculated the RfC from a no-observed-adverse-effect level (NOAEL) derived from animal studies. From the NOAEL, the EPA determined an equivalent human concentration and applied uncertainty factors to obtain the RfC of 5 $\mu g/m^3$. It is not the intention of this paper to conduct a similar exercise. Instead, we discuss some of the important issues that arise when conducting and interpreting such calculations.

## RESPONSES OF INTEREST

When assessing dose response, several criteria are important for deciding which endpoints should be examined: sensitivity and biological relevance should both be considered. Chronic inflammation and epithelial cell hyperplasia are two endpoints that are sensitive indicators of response and are probably biologically relevant to the lung pathology observed in chronically exposed rats. Chronic inflammation, with its associated mediators of fibrosis, oxygen free radicals, and mitogens, could be related to fibrotic and neoplastic processes (Driscoll et al. 1990; Sibille and Reynolds 1990; Bowden and Adamson 1994; see also Figure 2 of Oberdörster 1994 and the background paper by Busby and Newberne). Chronic alveolar inflammation is usually assessed by measuring the influx of inflammatory cells (alveolar macrophages and PMNs) into the airspaces, the presence of protein and enzymes in the bronchoalveolar lavage fluid, and the aggregation of particle-laden macrophages in the histologic preparations. Most of the focus has been on cells or molecules capable of either causing lung tissue damage or representing injured tissue. Measuring specific mediators will provide additional information on the lung's capacity to repair tissue damage caused by inflammatory cells. For example, Henderson and associates (1988b) analyzed lung tissue and bronchoalveolar lavage fluid for various cellular mediators relating to oxidative damage. They observed that eventual fibrosis was correlated with the lung tissues' capacity to deal with oxidative stress. In addition, assessment of events in the interstitium may provide useful information, especially with respect to pulmonary fibrosis.

Cell proliferation has been implicated in nongenetic mechanisms of carcinogenesis (Ames and Gold 1990; Cohen and Ellwein 1990; Preston-Martin et al. 1990). The mechanisms by which diesel exhaust exposure induces cellular proliferation and how such increases in cell replication relate to tumor formation are unknown; nonetheless, epithelial hyperplasia in rats is associated both with diesel exhaust exposure and with tumor formation. Although many factors may relate to the hamster's resistance to pulmonary carcinogenesis, it is interesting to note that hamsters appear to exhibit less of a hyperplastic response to

chronic diesel exhaust exposure than rats or mice. Heinrich and coworkers (1986) reported a bronchioloalveolar hyperplasia incidence rate of 64% in mice and 98% in rats, but only mentioned an "increase" in hamsters. In a later study by Heinrich and associates (1989), bronchiole-alveolar hyperplasia in hamsters exposed to diesel exhaust (3.7 mg/m$^3$, 19 hours/day, five days/week) was minimal until 18 months of exposure. A better understanding of nongenetic mechanisms of cancer or of the chronic disease process will aid our interpretation of the implications of these endpoints.

Impaired alveolar clearance is another effect that occurs when rats are exposed to diesel exhaust. (For more discussion on this phenomenon, see the background paper by Green and Watson.) When the deposition rate of inhaled particles exceeds the clearance capacities of the lungs, alveolar clearance rates decrease and deposited material begins to accumulate. One of the advantages of evaluating impaired alveolar clearance is that considerable quantitative information on dose parameters is available. Oberdörster (1995) has suggested that disturbed pulmonary clearance is necessary for the induction of fibrogenic and tumorigenic responses in rats exposed to particles of low cytotoxicity. In addition, impaired clearance in rats has been detected after only three months of diesel exhaust exposure, whereas other endpoints are more obviously apparent at later time points. Finally, reductions in lung clearance probably occur in humans chronically exposed to large quantities of insoluble particles; thus, extrapolating from this effect in rats to humans is reasonable. Oberdörster (1994) notes the greater difficulty in extrapolating fibrotic and tumorigenic reactions across species because of species-to-species differences in response to retained lung particles. The major shortcoming of using particle clearance as an endpoint is that we lack a good understanding of how impaired lung clearance relates to lung disease (Mauderly 1994a; Oberdörster 1994). For example, rats, mice, and hamsters exhibit prolonged particle clearance when exposed to "overloading" concentrations of various insoluble particles; however, fibrosis occurs to a lesser extent in mice and hamsters, and only rats develop lung tumors (Oberdörster 1995).

## DOSE RESPONSE

### Dose Parameters

Because of studies showing greater effects in animals exposed to unfiltered diesel exhaust than in those exposed to filtered exhaust (Heinrich et al. 1986; Iwai et al. 1986), diesel particles have been used as the basis of dose. Selection of the particulate fraction is reinforced by the observation of lung tumors in rats chronically exposed to particles other than diesel exhaust. Two major concerns are (1) the best way to express dose and (2) the determination of dose equivalence between rats and humans. Airborne concentrations of diesel particles, per se, are not adequate. Adjustments must be made for the duration of exposure and/or particle accumulation (i.e., lung burden).

Different approaches have been used to normalize exposure conditions among various studies. To adjust for differences in exposure duration, the EPA estimated "duration-adjusted concentrations"; to derive these concentrations, the EPA multiplied the ambient air concentration by the fraction of weekly exposure (U.S. Environmental Protection Agency 1993). For example, in Mauderly's study in 1986, when rats were exposed to 0.35, 3.5, or 7.0 mg/m$^3$ of diesel exhaust for seven hours/day, five days/week, the diesel particle concentrations were multiplied by 35/168 to obtain the duration-adjusted concentrations of 0.07, 0.74, or 1.47 mg/m$^3$. Another approach to normalizing different exposure protocols is to calculate integrated exposure per week. In this case, the ambient air concentration is multiplied by the total number of hours the animals were exposed to obtain an integrated particle exposure per week. Again, using Mauderly's study (1986) of a 35-hour exposure per week, we obtain weekly particle concentrations of 12.25, 122, or 245 mg/m$^3$ • hour.

Because of the importance of lung overload in rats (see Oberdörster 1995), interest in using the amount of retained particles in lung tissues (i.e., lung burden) as a measure of dose has been increasing. The primary advantage of lung burden measurements is that they take into account that dose is not uniform per unit of time or per unit ambient concentration; that is, at high ambient air concentrations, the dose of particles to lung tissues increases with duration of exposure. Often, mathematical estimates are used for lung burdens, or nonlinear dose, during risk assessment calculations; however, several recent studies (Wolff et al. 1987; Creutzenberg et al. 1990; Mauderly et al. 1989, 1994) have generated quantitative data on lung burden and provide such information when describing nonneoplastic effects. In these cases, experimental data can be used directly to analyze dose response.

### Correlation of Dose Parameters with Responses of Interest

Table 7 illustrates dose-response information for two parameters of chronic alveolar inflammation (i.e., influx of alveolar macrophages and PMNs). The number of alveolar macrophages in lavage fluid does not exhibit a consistent dose response and seems better correlated with the reporting investigator. This may be due to investigator-specific lung lavage procedures. In contrast, the influx of PMNs is a sensitive indicator of a response to diesel exhaust. Reasonably consistent increases can be observed for weekly

particle exposures greater than 80 mg/m$^3$ • hour. Because of the variability in cell numbers among different studies, the results summarized in Table 7 were also expressed as the ratio of cells in animals exposed to diesel exhaust to cells in control animals. These ratios were not dose related (data not shown).

Table 8 illustrates dose-response information for epithelial cell proliferation. Data in Table 8 show that below a weekly particle exposure of approximately 70 mg/m$^3$ • hour, epithelial cell proliferation is minimal. Study results also suggest that particles from heavy-duty diesel engines stimulate less of a proliferative response than particles from light-duty diesel engines. Only the study by Mauderly and coinvestigators (1994) provides detailed information on the onset, incidence, and severity of the proliferative responses. Using a "severity" scale, the authors observed that the lesions were more severe in animals exposed to higher particle concentrations, and the severity of the lesions increased with total duration of exposure.

Table 9 shows dose-response information for impaired clearance along with data for various dose parameters, including lung burden. The only data point for which the alveolar clearance half-time was not significantly different from control values was for a weekly exposure of 12.3 mg/m$^3$ · hour or a lung burden of 0.6 mg/lung (Wolff et al. 1987). Also, Mauderly and coworkers (1994) found that after three months of exposure to diesel exhaust or carbon black particles, impaired clearance was dose related; however, after 18 months of exposure, all exposed groups, regardless of exposure concentration or lung burden, experienced similar impairments in clearance.

Tables 7, 8, and 9 show that for each response there is a dose below which an effect of diesel exhaust exposure cannot be detected. This observation is supported by Mauderly's review of several nonneoplastic effects from a series of studies conducted at the Inhalation Toxicology Research Institute (Mauderly 1994b). He reported that at lung burdens of less than 0.5 mg/g of lung, no significant alterations were observed in histopathology, particle clearance, pulmonary function, or immunologic competence. Nonaffected rats were exposed to 0.35 mg/m$^3$, seven hours/day, five days/week, resulting in a weekly exposure of 12.3 mg/m$^3$ · hour.

Which descriptors of dose, normalized particle concentrations or lung burdens, correlate better with a dose response is unclear. As noted above, the recent study by

**Table 7.** Presence of Alveolar Macrophages and Polymorphonuclear Leukocytes in Bronchoalveolar Lavage Fluid from Rats Exposed to Diesel Exhaust for 12 Months

| Reference | Duration-Adjusted Particle Concentration (mg/m$^3$) | Weekly Particle Exposure (mg/m$^3$ • hour) | Alveolar Macrophages[a] | Polymorphonuclear Leukocytes[a] |
|---|---|---|---|---|
| Mauderly et al. 1994 | 3.12 | 520 | – | + |
| Heinrich et al. 1986 | 2.39 | 399 | + | +[b] |
| Henderson et al. 1988b | 1.47 | 245 | – | + |
| Mauderly et al. 1994 | 1.20 | 200 | – | – |
| Strom 1984 | 0.98 | 165 | + | + |
| Henderson et al. 1988b | 0.74 | 123 | – | + |
| Mauderly et al. 1989 | 0.74 | 123 | – | –[c] |
| Strom 1984 | 0.49 | 82.5 | + | + |
| Strom 1984 | 0.16 | 27.5 | – | – |
| Henderson et al. 1988b | 0.07 | 12.25 | – | – |

[a] – = No increase in cell numbers reported by author(s); + = increase in cell numbers reported by author(s).

[b] Data taken after 24 months of exposure; data not reported for 12 months.

[c] Control values were very high (i.e., 100 × 10$^3$ cells/mL of lavage fluid).

Mauderly and associates (1994) provides detailed information on the incidence, onset, and severity of nonneoplastic effects. They found that most responses could be correlated with particle exposure concentrations; however, for some effects, cumulative lung burden was better associated with a response. Reductions in body weight and increases in lung weight were correlated with exposure concentrations; incidences and severity of histopathologies correlated better with lung burden; and some bronchoalveolar lavage indicators of inflammation and cytotoxicity were related to both dose parameters. Interestingly, the elevation of PMNs, which is a sensitive response to diesel exposure, was not related to either exposure concentration or lung burden.

**Exposure Rate**

Exposure concentrations, duration adjustments, and lung burdens do not take into account the exposure rate, which conceivably could affect outcome. Using carbon black particles, Henderson and coinvestigators (1992) exposed rats for 12 weeks to three different exposure rates ("low" delivery rate, 3.5 mg/m$^3$, 16 hours/day, seven days/week; "medium" delivery rate, 13 mg/m$^3$, 6 hours/day, five days/week; and "high" delivery rate, 98 mg/m$^3$, 4 hours/day, one day/week). The three different protocols were designed to provide the same concentration × time product of particles to the lungs. They measured lung burdens, evaluated lung histopathology, and assessed various components of bronchoalveolar

**Table 8.** Presence of Epithelial Cell Hyperplasia in Rats Exposed to Diesel Exhaust for at Least 18 Months

| Reference | Duration-Adjusted Particle Concentration (mg/m$^3$) | Weekly Particle Exposure (mg/m$^3$ • hour) | Cell Proliferation (% of animals affected)[a] |
|---|---|---|---|
| Mauderly et al. 1994 | 3.12 | 520 | + (100) |
| Heinrich et al. 1986 | 2.39 | 399 | + (99) |
| Ishinishi et al. 1986 | 2.29[b] | 348[b] | + (20.02) |
| Iwai et al. 1986 | 1.62 | 274 | + |
| McClellan et al. 1986 | 1.47 | 245 | + |
| Mauderly et al. 1994 | 1.2 | 200 | + |
| Ishinishi et al. 1986 | 1.14[b] | 192[b] | + (11.4) |
| Ishinishi et al. 1986 | 1.14 | 192 | + (70.2) |
| Mauderly et al. 1989 | 0.74 | 123 | + |
| Ishinishi et al. 1986 | 0.57[b] | 96[b] | ± (<6) |
| Ishinishi et al. 1986 | 0.57 | 96 | + (9.8) |
| Lewis et al. 1986 | 0.42[b] | 70[b] | + |
| Ishinishi et al. 1986 | 0.23[b] | 57[b] | ± (<6) |
| Ishinishi et al. 1986 | 0.23 | 57 | ± (<5) |
| Ishinishi et al. 1986 | 0.06 | 23 | ± (<5) |
| McClellan et al. 1986 | 0.07 | 12.3 | – |

[a] + = Present; ± = minimal; – = not present.

[b] Diesel exhaust from a heavy-duty engine.

lavage fluid. Although lung burdens were approximately 20% higher in rats exposed to the low delivery rate, inflammatory effects and lung histology were similar in all three exposure groups. Thus, with carbon black particles, they did not find an effect of exposure rate on lung pathology.

### Particle Volume

Most analyses have used gravimetric measures as the basis for dose. In addition, particle volume may be relevant. Given the hypothesis relating macrophage volumetric load with impairment of alveolar-macrophage–mediated clearance (Morrow 1988), particle volume may be the most appropriate dose parameter to use when evaluating lung clearance. When analyzing several studies using different insoluble particles, Morrow and associates (1991) found that the pulmonary clearance coefficient, $k$, correlated with retained dust volume (see Figure 2 in their article, or Figure 4 in the background paper by Green and Watson).

### Particle Surface Area

In addition to particle mass and particle volume, some evidence indicates that particle surface area may be a critical factor when assessing dose-response relationships (Oberdörster 1994). Inflammation (Oberdörster et al. 1995) and tumor incidence (Oberdörster and Yu 1990; Heinrich 1994) have been suggested to correlate with the surface areas of various insoluble particles retained in the alveolar spaces. Using two different-sized titanium dioxide particles ("ultrafine," primary

**Table 9.** Alveolar Clearance in Rats Exposed to Diesel Exhaust for 3 or 18 Months

| Reference | Duration-Adjusted Particle Concentration ($mg/m^3$) | Weekly Particle Exposure ($mg/m^3$ • hour) | Lung Burden at 3 Months (mg/lung)[a] | Half-Time of Clearance in Days (ratio of clearance in exposed rats: clearance in control rats)[a] | Lung Burden at 18 Months (mg/lung)[a] | Half-Time of Clearance in Days (ratio of clearance in exposed rats: clearance in control rats)[a] |
|---|---|---|---|---|---|---|
| Creutzenberg et al. 1990 | 4.28 | 713 | 8.2 | 330* (5.4) | 58.8 | 687* (7.2) |
| Mauderly et al. 1994 | 3.12 | 520 | 5.9 | 1,157*[b] (33.1) | 49.6 | —[c] |
| Heinrich et al. 1986 | 2.39 | 399 | ND | ~ 170* (2.8) | ND | ~ 120*[d] (2.7) |
| Wolff et al. 1987 | 1.47 | 245 | 2.7 | ND | 20.5[e] | 240*[e] (3.0) |
| Creutzenberg et al. 1990 | 1.43 | 238 | 2.5 | 119* (2.0) | 12.8 | 272* (2.8) |
| Mauderly et al. 1994 | 1.2 | 200 | 2.63 | 62* (1.8) | 25.5 | 987* (18.0) |
| Wolff et al. 1987 | 0.74 | 123 | 1.2 | ND | 11.5[e] | 264*[e] (3.3) |
| Mauderly et al. 1989 | 0.74 | 123 | ND | ND | 6.86 | 109* (2.1) |
| Creutzenberg et al. 1990 | 0.46 | 76 | 0.63 | 74* (1.5) | ND | 221* (2.3) |
| Wolff et al. 1987 | 0.07 | 12.3 | 0.13 | ND | 0.60[e] | 81e (1.0) |

[a] * = Significantly different from value in control animals; ND = not determined.

[b] Half-time calculated from Table 11 of Mauderly et al. 1994.

[c] Clearance of tracer particles to 50% of initial lung burden was never reached.

[d] Data taken after 19 months of diesel exhaust exposure.

[e] Data taken after 24 months of diesel exhaust exposure.

particle diameter = 0.02 μm, particle surface area = 50 $m^2/g$; "fine," primary particle diameter = 0.25 μm, particle surface area = 6.4 $m^2/g$), Oberdörster and coworkers (1995) compared the retention and inflammatory response in particle-exposed rats; the exposure protocol was such that similar mass depositions were achieved for the two different particles. The investigators found a greater inflammatory response and translocation to the interstitium with the ultrafine particles than with the fine particles. Also, pulmonary clearance of the ultrafine particles was slower than that of the larger material. Furthermore, the authors reported that, with the ultrafine particles, volumetric load of the alveolar macrophages was not a good dose determinant for assessing delayed clearance. Differences in effects between the two particle types could be expressed with a common dose-response relationship if particle surface was used as the dose term. Because of its porous structure, the surface area of diesel exhaust particles is reasonably large (50 to 200 $m^2/g$ of soot or 30 to 50 $m^2/g$ of elemental carbon core). If the data exist, the surface area of other particles should be considered when comparing the effects of diesel exhaust with those of other insoluble particles. In addition, if particle characteristics change with alterations in engine design and fuel characteristics, both surface area and particle size should be considered as important components of exposure characterization.

## QUANTITATION OF RESPONSE

Although the discussions above focused on the quantitation of dose, consideration must also be given to quantitation of response. For some endpoints, such as alveolar clearance, numerical information is available or can be calculated from clearance curves. Statistical comparisons can then be made with nonexposed animals. Other endpoints are not as amenable to analytical treatment. For example, most investigators qualitatively describe the occurrence of epithelial hyperplasia in histologic sections from animals exposed to diesel exhaust. In some studies (Heinrich et al. 1986; Iwai et al. 1986; Mauderly et al. 1994), the percentage incidence of hyperplasia was provided; however, morphometric measurements or some numerical scale of severity (Mauderly et al. 1994) provides additional, important information for quantitation of response.

Quantitation of response also allows for some analysis of the threshold of response; that is, with appropriate quantitation of both axes of the dose-response graph, the shape and the slope of the curve can be determined. Shape and slope are parameters extremely helpful for evaluating species sensitivity and thresholds and for extrapolating across species.

## EXTRAPOLATION FROM RATS TO HUMANS

The rat is the species most sensitive to diesel exhaust exposure and is the species that regulatory agencies use in extrapolation modeling. Dose-response curves obtained in rats are extrapolated to human exposure conditions using mathematical models (described in the background paper by Green and Watson). It is assumed, however, when extrapolating from the response observed in animals, that humans will experience the same response at equivalent doses (U.S. Environmental Protection Agency 1993). We do not know if this is true. Thus, although inflammation and cell proliferation occur in rats chronically exposed to weekly diesel particle concentrations greater than 70 to 80 $mg/m^3 \cdot$ hour, we do not know the relative sensitivity of humans to equivalent doses of diesel particles in the lungs. We can predict equivalent lung doses with some associated uncertainty, but far greater uncertainty probably resides in species-to-species responses to the predicted doses.

The work of Henderson and coworkers (1988b) clearly demonstrated that species differ in their response to diesel exhaust inhalation, even when parameters of dose are similar. Rats and mice were exposed to identical airborne concentrations of diesel exhaust, resulting in lung burdens that were slightly higher in mice. To assess the antioxidant defense system, the investigators measured glutathione and glutathione reductase in the bronchoalveolar lavage fluid and lung tissue from rats and mice exposed to diesel exhaust. Mice had greater amounts of glutathione in their lavage fluid and lung tissue and higher levels of glutathione reductase in their lavage fluid than rats. Furthermore, levels of lung tissue glutathione increased with exposure in mice but decreased in rats. These data suggest that mice are more capable of accommodating an oxidative stress than rats, even though exposure was identical and lung burdens were slightly higher in the mice. In addition, the mice exposed to diesel exhaust exhibited less epithelial cell hyperplasia, inflammation, and focal fibrosis than the exposed rats.

Mauderly (1994b) reviewed Heinrich and coworkers' study (1986) and compared nonneoplastic effects in identically exposed rats and hamsters. Mauderly noted that indicators of cytotoxicity and inflammation in the hamsters, as measured in the bronchoalveolar lavage fluid, were approximately half those in the rats.

The appropriateness of the rat model for human responses is an issue that has been raised on several occasions in the context of lung tumor formation in response to chronic particle inhalation (Dungworth et al. 1994; Mauderly 1994a; Oberdörster 1994). If the reported nonneoplastic effects are related mechanistically to lung tumor formation in rats, the relevance of such effects in rats to health effects in humans

must be similarly questioned. Alternatively, we perhaps can use the presence of these nonneoplastic effects to gain insight into mechanisms and base additional work on exploring species differences in carcinogenesis at an earlier time of response, instead of waiting until tumors develop. Even apart from any role of nonneoplastic responses in lung tumor formation in rats, extrapolation of these responses to noncancer endpoints in humans is uncertain.

## CONCLUSIONS

We have reviewed approximately 50 reports describing noncancer effects in animals from the inhalation of diesel exhaust. We have correlated these effects with different descriptors of dose and discussed some of the major issues that should be considered when extrapolating a dose response observed in animal studies to one predicted in humans. From the data reported in the animal studies and our dose-response discussions, we conclude the following:

- Animals exposed to diesel exhaust exhibit a number of nonneoplastic pulmonary effects, including chronic inflammation, epithelial cell hyperplasia, metaplasia, alterations in connective tissue, pulmonary fibrosis, and compromised pulmonary function.
- Based on the proportion of animals affected and the dose at which responses are reported, rats are the most sensitive species tested.
- Chronic inflammation and epithelial cell hyperplasia are probably the two most sensitive effects of biological significance. Impaired alveolar clearance in rats exposed to diesel exhaust also is an important endpoint, but its relationship to disease processes in other species is unclear.
- These endpoints may be related mechanistically to the tumors observed in rats chronically exposed to diesel exhaust and other insoluble particles. Mediators from inflammatory cells may also contribute to the pulmonary fibrosis observed in rats exposed to diesel exhaust.
- Numerous ways of quantifying dose are available, and it is unclear if integrated exposure or cumulative lung burdens are better descriptors. Some evidence indicates that nongravimetric measures, such as particle surface area or particle volume, may be more appropriate for certain endpoints, such as indicators of inflammation or decreased alveolar clearance.
- Weekly exposures to diesel exhaust of 70 to 80 $mg/m^3 \cdot$ hour or greater are associated with the presence of chronic inflammation, epithelial cell proliferation, and depressed alveolar clearance in chronically exposed rats. The occurrence of these effects also is associated with lung burdens of diesel soot of 0.5 mg/g of lung or greater. Below these dose levels, an effect of diesel exhaust exposure is not detectable.
- The quantitation of response is equally important, and more rigorous efforts in quantifying response would provide a more informative dose-response analysis.
- The major limitation in dose-response exercises resides in the extrapolation from animal models to humans. Conditions of human exposure can be simulated mathematically, but the characterization of human responses, or biological susceptibility, is the greatest obstacle in human health assessment.

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## ABBREVIATIONS

| | |
|---|---|
| EPA | U.S. Environmental Protection Agency |
| Ig | immunoglobulin |
| NOAEL | no-observed-adverse-effect level |
| PMNs | polymorphonuclear leukocytes |
| RfC | reference concentration |

# Relation Between Exposure to Diesel Emissions and Dose to the Lung

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*Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects*

## PREAMBLE

Six principal issues underlie the interpretation of epidemiologic and toxicologic data suggesting the existence of health hazards from exposure to diesel exhaust: (1) extrapolation from effects observed at high doses to those possible at low doses, (2) extrapolation from effects in laboratory animals to effects in humans, (3) extrapolation from mechanisms of action observed in animal models to mechanisms in human disease, (4) interpretation of mechanistic data obtained in cellular and molecular studies for their significance in intact animals and humans, (5) relation of air concentrations measured in the environment to actual dose to target receptors under different conditions of exposure, and (6) extrapolation of results from acute conditions of exposure to chronic. These issues are critical in the evaluation of diesel exhaust as a human pathogen at ambient levels.

Animals exposed to diesel exhaust show neoplastic and nonneoplastic effects in the lungs. Cancer has been consistently produced experimentally only in rats that received very high levels of exposure and massive doses to the lungs (Figure 1; also see background paper by Busby and Newberne, this report). Cancer has not been produced in hamsters and has been produced only inconsistently in mice. A serious question remains as to whether the mechanism of carcinogenicity at these high doses in animals is the same mechanism of action in humans at much lower doses.

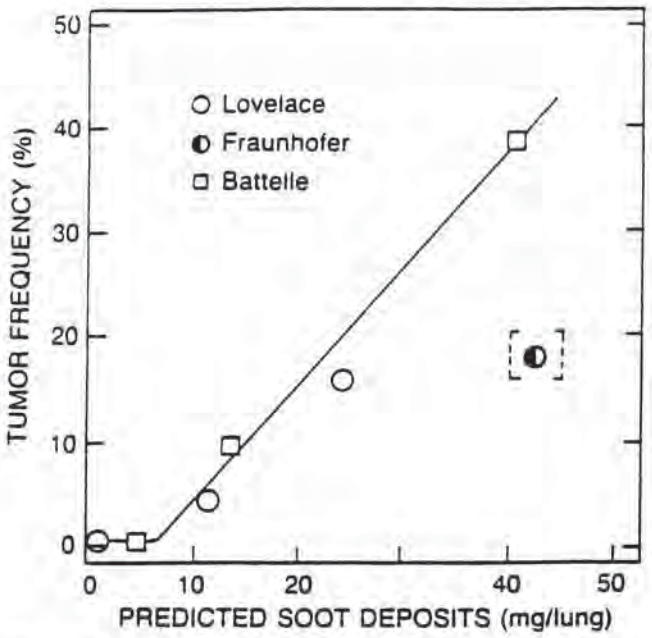


**Figure 1. The relation between the predicted diesel exhaust particle (soot) deposits in the lung and the frequency of tumors in diesel inhalation studies.** (Reprinted from Vostal [1986], with permission of the editors.)

Epidemiologic data suggest a cancer effect in occupational groups at the upper end of the human exposure spectrum (Figure 2; also see background paper by Cohen and Higgins, this report). Toxicologic effects observed in animals exposed to diesel exhaust may cause the pulmonary fibrosis reported in some studies. These effects also may be related in some way to the mechanism of carcinogenesis. Therefore, extrapolation of animal data to the assessment of risk in the general human population will depend on (1) confirmation that the biologic mechanisms are closely similar in susceptible animal species and in humans, (2) that the dose-response curves are comparable, and (3) that ambient exposure levels are sufficient to produce an effective dose in the human lung.

In this paper we describe the components of host defense that influence the translation of what is measured in the environment as "exposure" to what reaches the target tissue as "effective dose." Our focus is on determining what fraction of exposure is converted to dose. We analyze the components of deposition, distribution, bioavailability, clearance, translocation, and retention and how dose to pulmonary tissues is affected by these processes in different animal species. We consider the location of target cells where biologic activity has been identified. We discuss how exposure conditions affect the distribution of dose. Finally, mathematical models that are used to predict exposure and dose and their extrapolations to humans are described.

## EFFECTS OF SPECIES ON PARTICLE DEPOSITION, CLEARANCE, AND RETENTION

A comprehensive review of deposition, clearance, and retention of inhaled particles, with comparative informa-

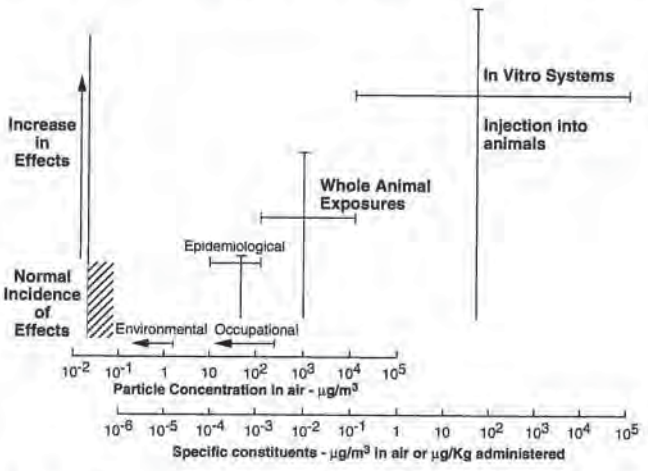


**Figure 2. Exposure-response relations relevant to evaluating the cancer risks of diesel exhaust.** (Reprinted from McClellan [1986], with permission of the editors.)

tion in different animal species, can be found in Oberdörster (1988), Schlesinger (1988), and Snipes (1989). Diesel exhaust particles are respirable, meaning that they are sufficiently small (0.1 to 0.3 μm) (Frey and Corn 1967) to penetrate the aerodynamic defenses of the upper respiratory tract and deposit on the epithelia of the respiratory bronchioles and the alveoli. The behavior of diesel exhaust particles in the lungs is fairly well characterized (Mauderly et al. 1982, 1988, 1994; Strom 1984; Strom et al. 1990), and their fate is similar to that of highly insoluble particles. In general, insoluble particles deposited on ciliated epithelium are swept centripetally to the trachea and hypopharynx by the mucociliary apparatus. Particles deposited on the respiratory epithelium are ingested by alveolar macrophages (see Sorokin and Brain 1974), which are then transported by respiratory tract fluid flow to the ciliated epithelium of the bronchioles or to peribronchial and perivascular areas (Green 1973); alternatively, particles become sequestered in macrophage maculae (Strom et al. 1990). When the concentration of inhaled particles is high, particles penetrate the alveolar epithelium and lodge in the interstitium, where they may in turn be taken up by tissue macrophages (Adamson and Bowden 1981) and become transported to subpleural and lymphatic focal points of long-term storage. These removal processes are collectively referred to as pulmonary clearance.

Although available evidence indicates that diesel exhaust particles are deposited, cleared, and stored like other insoluble particles, a prominent characteristic of diesel particles is the adsorption of potentially toxic organic compounds from the volatile fraction of the combustion mixture. The quantity of particles, with adsorbed organic compounds, residing at any point in the respiratory tract becomes the initial dose to that locus. Consideration also must be given to the effects of oxidant and irritant gases such as nitrogen dioxide, which are an integral component of the exposure to whole diesel exhaust. The recruitment and proliferation of pulmonary macrophages in response to a particulate challenge (Adamson and Bowden 1981) serves to increase the defensive capacity of the alveolar surface, reduce the dose to the epithelial cells, and decrease the penetration of particles to the interstitium. Quantitative extrapolation of these kinetics from animals to humans must consider species differences in the variables of deposition, clearance, and host responses, including elution of adsorbed organic compounds and retention, as they affect dose to target cells (Lippmann and Schlesinger 1984).

## DEPOSITION

The first point to be explored concerns the differences among species in the fraction of inhaled particle concentration that penetrates the upper respiratory tract and becomes deposited on the ciliated epithelium of the conducting airways and on the epithelium of the peripheral respiratory tissue and alveolar cells (the deposition efficiency). In a detailed review, Lippmann and Schlesinger (1984) summarized studies comparing interspecies differences in the tracheobronchial deposition of various insoluble nondiesel particles ranging from 0.28 to 7.0 μm in humans and in the dog, donkey, hamster, rabbit, and rat (Table 1); they then compared the distribution of deposited particles among the different divisions of the tracheobronchial tree in a hollow cast of the human airway. Fractional tracheobronchial deposition generally was similar among these species (lowest in the dog) and increased by 2- to 10-fold with increasing particle size (0.8 to 6.0 μm).

In studies of diesel exhaust in which particle sizes are nearly an order of magnitude smaller, Chan and coworkers (1981) found that 15% to 17% of the inhaled particles were deposited in the lower respiratory tract of the rat at particle sizes of 0.1 to 0.19 μm; Lee and associates (1983) estimated essentially identical fractional deposition (17% to 20%) using particles of 0.12-μm diameter. Although rats were exposed to different exposure regimens (2 $mg/m^3$ for 140 minutes or 7 $mg/m^3$ for 45 minutes), deposition efficiency was comparable. Guinea pigs showed a fractional deposition similar to that of rats (Table 2). Data reported by Chan and Lippmann (1980), Cuddihy and associates (1973), and Stahlhofen and colleagues (1980) show that dogs and humans experience comparable fractional deposition of diesel exhaust–sized particles. In humans, it has been shown that 0.2-μm particles deposit predominantly in the alveolar region, with an efficiency of 13% to 21%; bronchial deposition of particles of this size was undetectable by methods used (Heyder et al. 1986). Proposed changes in diesel fuel characteristics (e.g., sulfur content) or changes in engine design might affect particle size distribution. If particles become smaller, deposition efficiency can be expected to rise from an increase in diffusional forces. Deposition efficiency also will increase if particles become larger because of the greater influence of gravimetric forces and impaction.

Previous exposure to small-sized particles does not seem to affect deposition efficiency. Deposition of an indicator particle was not affected by previous exposure to diesel exhaust even under overload conditions (Table 3).

Thus, for diesel particles, deposition efficiency, which is governed by the physical principles of inertia, gravitation, and diffusion, is reasonably consistent across species, age, and level of previous exposure.

## CLEARANCE

It can be assumed that the clearance kinetics of diesel exhaust particles from the respiratory tract are similar to those of other insoluble particles of comparable size, shape, and density. After an acute exposure to diesel engine exhaust, the removal of particles from rat pulmonary surfaces follows a biphasic pattern, with removal half-lives of 1 day and 62 days (Chan et al. 1981). This biphasic clearance pattern, and observations from morphologic studies (Strom et al. 1990), suggest that mucociliary transport and macrophage-mediated transport, respectively, are the likely predominant clearance mechanisms.

In contrast to the relative comparability across species of fine particle deposition, clearance of insoluble particles, including diesel particles, from the distal bronchioles and alveoli varies markedly among species with the rate of dust accumulation and the extent of previous exposure. The clearance half-life of material deposited in the alveolar region ranges from weeks to months, depending on the route of exit from the lung. If material becomes sequestered in alveolar, parabronchial, or paravascular maculae or reaches pulmonary lymph nodes, the residence time increases from months to years.

Pepelko (1987) and Snipes (1989) reviewed long-term clearance data for various particles in hamsters, guinea pigs, mice, and rabbits exposed to diesel exhaust and other particles. Studies in dogs and in humans showed a wide range of long-term clearance rates related to the composition of the particle, but the data did not include findings with diesel exhaust particles; for nondiesel particles, alveolar clearance rates were considerably longer (several hundred days) in dogs and in humans than in rodents. For diesel exhaust particles, half-time clearance rates are simi-

**Table 1.** Tracheobronchial Deposition of Inhaled Particles in Various Species[a]

| Species | Aerodynamic Diameter (μm) | Percent of Total Lung Deposition Occurring in the Tracheobronchial Tree | | | Reference |
|---|---|---|---|---|---|
| | | Median | Mean | Range | |
| Human | 0.8 | 19 | — | 8–29 | Chan and Lippmann 1980 |
| | 3.0 | 30 | — | 10–57 | Lippmann 1977 |
| | 6.0 | 65 | — | 28–92 | Lippmann 1977 |
| Dog | 0.5–0.6 | ~4 | — | — | Cuddihy et al. 1973 |
| | 2.5–3.0 | ~11 | — | — | Cuddihy et al. 1973 |
| | 6.6 | ~44 | — | — | Cuddihy et al. 1973 |
| Donkey | 0.3 | 25 | — | 18–33 | Schlesinger 1975 |
| | 3.0 | 58 | — | 49–68 | Albert et al. 1968 |
| | 5.0 | 70 | — | 68–75 | Schlesinger and Lippmann 1978 |
| | 5.0 | 64 | — | 55–70 | Schlesinger and Lippmann 1979 |
| Hamster | 0.5 | — | 6 | — | Raabe et al. 1977 |
| | 3.1 | — | 25 | — | Raabe et al. 1977 |
| Rabbit | 0.5 | 33 | — | 20–64 | Leikauf 1978 |
| | 3.0 | 29 | — | 17–55 | Holma 1967 |
| | 4.5 | 72 | — | 66–79 | Chen and Schlesinger 1983 |
| | 6.0 | 60 | — | 40–93 | Holma 1967 |
| | 6.0 | 77 | — | 54–80 | Leikauf 1978 |
| Rat | 0.5 | — | 5 | — | Raabe et al. 1977 |
| | 3.1 | — | 50 | — | Raabe et al. 1977 |

[a] From Lippmann M, Schlesinger RB, 1984, J. Toxicol Environ Health 13:450, Table 1, Taylor & Francis, Inc., Washington, DC. Reproduced with permission. 

**Table 2.** Experimental Deposition of Diesel Engine Exhaust Particles in the Respiratory Tract[a]

| Species | Mass Median Particle Diameter (μm) | Percentage of Total Deposition of Inhaled Exhaust Particles | Reference |
|---|---|---|---|
| Rat | 0.1–0.15 | 15–17 | Chan et al. 1981 |
| Rat | 0.16–0.19 | 10–17 | Dutcher et al. 1984 |
| Rat | 0.12 | 17[b] (Calculated), 20[b] (Estimated) | Lee et al. 1983 |
| Guinea pig | 0.12 | 20[b] (Initial deposition) | Lee et al. 1983 |

[a] Reprinted with permission from the International Agency for Research on Cancer, as published in International Agency for Research on Cancer (1989).
[b] Mean values.

lar in rats, but range from 62 to 87 days (Chan et al. 1981; Griffis et al. 1983; Lee et al. 1983; Wolff et al. 1987). In contrast, Lee and coworkers (1983) measured clearance in guinea pigs exposed during the same experiments as rats (see above) and observed virtually no alveolar-phase clearance of diesel exhaust particles in the guinea pigs (Figure 3).

Thus, clearance rates for inhaled particles show wide interspecies and intraspecies variability, making extrapolation across species somewhat problematic. Furthermore, previous lung burden slows lung clearance, although the degree and the duration differ in different experiments (see below).

## RETENTION

The net difference between deposition and clearance at any time following exposure is called retention. Snipes and coworkers (1983) compared the retention of aluminosilicate particles (0.7, 1.5, or 2.8 μm) in dogs, rats, and mice. Although these particles are soluble in biologic fluids over time, the investigators showed that retention was similar in rats and mice, but much more prolonged in dogs, monkeys, guinea pigs, and humans. Using diesel exhaust particles, Lee and associates (1983) showed that in rats retention was comparable at different exposure rates and concentrations as long as the total dose to the lung was the same. Strom and coworkers (1990), however, found that low concentrations of particles given over a long period resulted in more retention than high concentrations administered for brief periods. Griffis and associates (1983) showed that at high exposure rates the proportion of inhaled particles retained in the lungs of the rat doubled (Table 4). In contrast, Oberdörster and associates (1984) demonstrated that exposure to a diesel exhaust particle concentration of 2 mg/m$^3$ actually reduced retention of iron oxide particles even in the presence of a coal dust burden (Table 5), although the iron oxide was administered after only two months of exposure to the diesel exhaust. Thus, because alveolar, more than tracheobronchial, clearance is influenced by individual variation, species differences, and the rate at which the particles are delivered, the proportion of particles retained in the lungs varies widely. Furthermore, many questions regarding the effects of particle loading on clearance and long-term retention remain unanswered.

**Table 3.** Fractional Deposition of Tracer-Labeled Particles After Exposure to Diesel Exhaust Particles[a]

| Particle | Exposure Time (months) | Deposition (% of inhaled activity, $\bar{x}$ = SE) | | | |
|---|---|---|---|---|---|
| | | Control | Low (0.35 mg/m$^3$) | Medium (3.5 mg/m$^3$) | High (7.0 mg/m$^3$) |
| Gallium oxide ($^{67}Ga_2O_3$) | 6 | 12.2 ± 1.3 | 13.4 ± 1.9 | 9.9 ± 2.0 | 10.4 ± 1.3 |
| | 12 | 8.8 ± 1.3 | 12.7 ± 2.0 | 9.7 ± 1.4 | 10.5 ± 1.6 |
| | 18 | 7.0 ± 1.9 | 10.3 ± 2.5 | 13.9 ± 3.1 | 10.7 ± 1.9 |
| | 24 | 8.7 ± 1.3 | 14.2 ± 3.0 | 13.0 ± 2.5 | 11.1 ± 2.1 |
| Cesium ($^{134}$Cs-FAP) | 24 | 6.6 ± 1.7 | 7.7 ± 1.9 | 5.4 ± 1.8 | 8.1 ± 1.7 |

[a] Reprinted with permission from Academic Press and R.K. Wolff, as published in Wolff et al. (1987).

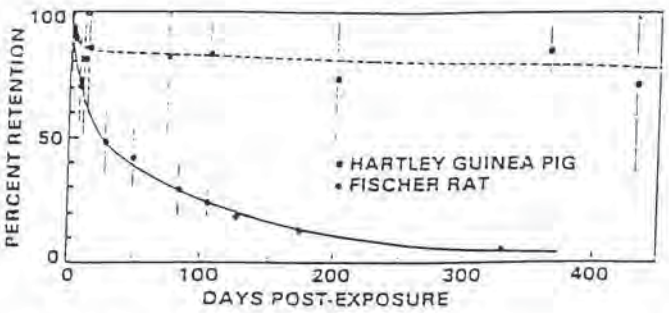


**Figure 3. Lung clearance of inhaled diesel exhaust particles from Fischer rats and Hartley guinea pigs after 45 minutes of nose-only exposure to a concentration of 7 mg/m$^3$.** The vertical line indicate SDs. (From Lee PS, Chan TL, Hering WE. 1983. J Toxicol Environ Health 12:80–813, Taylor and Francis, Inc., Washington, DC. Reproduced with permission. All rights reserved.)

## LUNG BURDEN AND EFFECTS ON CLEARANCE

While retention is a proportionate measure expressing that fraction of deposited particles not cleared by the lung, lung burden expresses the total mass of material found in the lungs at any point in time. It has long been recognized from studies of patients with pneumoconioses that lung burdens of mineral dusts in the working environment increase with higher exposures. Extensive animal studies have confirmed this relation. For example, Lee and coworkers (1987) showed a direct correlation between duration of exposure and lung burden for both diesel exhaust and carbon black particles at air concentrations of 6 mg/m$^3$, with the carbon black accumulating more rapidly than diesel exhaust particles (see Figure 6 from their reference). Mauderly and coinvestigators (1994) observed similar correlations of lung content of particles with exposure duration; however, in their study, diesel exhaust particles accumulated faster than carbon black particles, possibly due to their deeper penetration into the lung.

Lung overload occurs when clearance processes are overwhelmed by the amount of particles deposited over extended periods of time, which in turn results in lung burdens greater than what would have been predicted from deposition kinetics related to low exposure concentrations. Several investigators have reported that in rats lung burdens of diesel exhaust particles of 0.6 to 0.7 mg or greater per lung depress alveolar clearance (Griffis et al. 1983; Chan et al. 1984; Lee et al. 1987; Wolff et al. 1987; Creutzenberg et al. 1990; Mauderly et al. 1994), although no effect on rapid-phase (tracheobronchial) clearance has been detected (Wolff et al. 1987). Data from Pepelko (1987), however, showed that lower dose rates may provide partial protection against impaired clearance even at substantially higher lung burdens. Data for test toner (summarized by Morrow et al. 1991) show a sigmoid curve effect for clearance rate and suggest a threshold effect of burden on clearance at about 100 to 1,000 μg for particles of unit density in rat lungs. Impaired clearance of particles resulting from lung overload has been observed in rats, mice, hamsters, and dogs (Oberdörster 1994). The evidence of impaired clearance in humans is circumstantial. Lung burden and magnetopneumographic measurements in coal miners suggest that pulmonary clearance functions are impaired when workers are chronically exposed to high particle concentrations (Oberdörster 1994).

One theory to explain the slowing effect of particle burden on clearance is that the movement of the phagocytizing

**Table 4.** Lung Burdens of Diesel Exhaust Soot in Rats One Day After an 18-Week Exposure to Diluted Exhaust[a]

| Exposure Group | Average Net Aerosol Concentration[b] (μg/m$^3$) | Predicted Lung Burdenc (μg/g lung) | Net Lung Burden of Soot[d] (μg/g lung ± SD, $n$ = 8) | Lung Burden Normalized for Exposure Concentratione ([μg/g lung]/[μg/m$^3$]) |
|---|---|---|---|---|
| Control | 0 | 0 | | |
| Low | 150 | 47 | 35 ± 8 | 0.23 |
| Medium | 940 | 290 | 220 ± 38 | 0.23 |
| High | 4,100 | 1,280 | 1,890 ± 330 | 0.46 |

[a] Reprinted with permission from the Society of Toxicology and Griffis et al. (1983).

[b] Particulate levels found in the control chambers have been subtracted from total particulate levels.

[c] Estimated from deposition of gallium-67-labeled diesel particles in rats (Wolff et al. 1981).

[d] SD = standard deviation.

[e] Net lung burden divided by average aerosol concentration.

alveolar macrophages is retarded by the massive intracellular load of particles (Morrow 1988). Morrow (1988) postulated that the volumetric load of ingested particles by macrophages is the critical factor in lung clearance. Using data from various studies of dusts of different densities, Morrow and colleagues (1991) plotted pulmonary clearance coefficients on the basis of calculated values of particle volume. Figure 4 shows a linear relation plotted in this study between clearance and the volume of retained particles from 100 to 10,000 nL, at which point clearance comes to a halt. If on average 6% of the alveolar macrophage volume is taken up by particles, alveolar clearance starts to slow; at 60% cell volume, clearance stops. Results from Oberdörster and coworkers (1992) support Morrow's hypothesis of volumetric loading. After instillation of 10.3-μm polysterene particles, which would occupy approximately 60% of the macrophage cell volume, pulmonary clearance essentially ceased; clearance of 3.3-μm particles, administered in equivalent mass to that of the larger particles, was unaffected. The significance of Morrow's hypothesis is that it unifies results using widely different materials and indicates that the putative threshold for biologic effects may be at lower levels than previously believed.

In addition to the impact of large-particle volume on macrophage-mediated clearance, obstruction of exit pathways for particle-filled cells may contribute to delayed clearance. In rat lungs heavily exposed to titanium dioxide, Lehnert and coworkers (1994) observed occlusion of the pores of Kohn by alveolar septal thickening attributable to epithelial hyperplasia. Although the function of patent pores of Kohn is poorly understood, Green (1973) first suggested that the pores may decrease the distance alveolar macrophages need to travel when exiting distal alveoli.

**Table 5.** Relative Retention of Iron Oxide ($^{59}Fe_3O_4$) Particles in Rats During Exposure to Coal Mine Dust and Diesel Exhaust Particulate Matter (2 mg/m$^3$)[a]

| Group | Day 1[b] | Day 120 |
|---|---|---|
| Control | 88 ± 5 | 19 ± 4[c] |
| Diesel exhaust | 86 ± 6 | 8 ± 5[c] |
| Coal dust | 80 ± 10 | 14 ± 2[c] |
| Diesel + coal | 89 ± 15 | 13 ± 3 |

[a] Reprinted from Oberdörster G, Green FHY, Freedman AP, copyright 1984, Clearance of $^{59}Fe_3O_4$ particles from the lungs of rats during exposure to coal mine dust and diesel exhaust, J Aerosol Sci 15:235–237, with kind permission from Elsevier Science Ltd. (The Boulevard, Langford Lane, Kidlington 0X5 1GB, UK).

[b] Day 0 = 100%. Values are given as means ± SD.

[c] Significantly different groups indicated by boxes ($p < 0.05$).

Another locus of retained particles is in the hilar and tracheobronchial lymph nodes. Chan and colleagues (1981) found 6% of the initial lung deposition in the lymph nodes at four weeks after exposure. Using carbon black, Strom and coworkers (1989) found less in the nodes immediately after exposure, but when the threshold level for lung burden effects on clearance was exceeded, lung clearance decreased by 50% whereas lymphatic retention increased. Ferin and Feldstein (1978) showed similar results for titanium dioxide particles. In chronic exposure studies, Mauderly and associates (1994) observed the progressive accumulation of diesel exhaust particles and carbon black particles in the lung-associated lymph nodes of rats. It appears that the relative proportion of particles retained in lung-associated lymph nodes is inversely related to exposure concentrations. Mauderly and coworkers (1994) found that after 23 months of exposure the proportion of lymph node burden to lung burden was 5% for animals exposed to 2.5 mg/m$^3$ and 3.6% for animals exposed to 6.5 mg/m$^3$. Creutzenberg and coinvestigators (1990) measured proportions of 13%, 23%, and 28% in animals after 24 months of exposure to 7.5, 2.5, and 0.8 mg/m$^3$, respectively.

Lung burden, or the amount of diesel exhaust particles retained in the lungs, has been put forward as the most important determinant of response to a given exposure (McClellan 1990). Associated with increased lung burdens are pathophysiologic changes that include increased macrophage aggregation, altered cellular function and cytokine secretion, inflammation, pulmonary fibrosis, and possibly an increased incidence of lung tumors. The "dose" of ultimate interest, however, may be that concentration of diesel particles that elicits a specific cellular response for a given

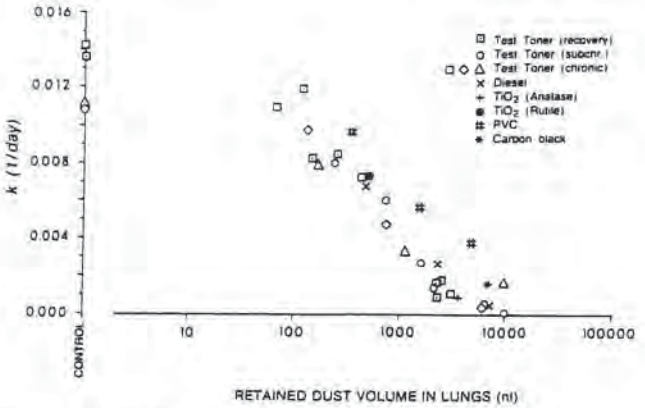


**Figure 4. Pulmonary clearance coefficients as a function of retained particle volume.** These data demonstrate the general finding that the alveolar clearance rates for a variety of dusts become significantly reduced at pulmonary dust volumes approaching 1,000 nL (i.e., the volume occupied by 1 mg of unit density material). (Reprinted with permission from The American College of Toxicology, as published in Morrow PE, Muhle H, Mermelstein R, 1991, J Am Coll Toxicol 10:279–290.)

animal species (Mauderly et al. 1987; see also Oberdörster and Pepelko 1992 and Oberdörster 1994). For example, Henderson and coworkers (1988) reported that with identical exposure regimens, mice accumulated a greater lung burden of diesel exhaust particles than rats; however, some cellular responses, such as fibrotic lesions, were greater in rats. Mauderly and coworkers (1987) also showed that after six months of exposure, developing rats had lung burdens of diesel exhaust particles (as expressed in mg/g of lung tissue) similar to lung burdens in adult animals; however, toxic effects were less severe in the developing animals.

In summary, alveolar deposition of fine particles, such as diesel soot, is relatively unaffected by species or by previous exposure. Clearance, however, differs across species and is retarded by previous particle burden (Morrow et al. 1991). In chronic exposure at milligram concentrations, clearance is retarded by a high dose rate (Bellmann et al. 1983), whereas at microgram levels, clearance is more efficient with high dose rates (Strom et al. 1990). Thus retention, which varies inversely with rate of clearance, differs across species, is greater in previously or simultaneously exposed lungs (possibly above an identifiable lung burden), and may be a principal variable in risk assessment considerations. Although lung burden, or some resultant biologic effect, may be a quantitative indicator of dose, the important biologic variable is clearance, because it determines retention and lung burden. The structural, biophysical, and functional properties that determine its efficiency, however, need clarification.

## PARTICLE-ASSOCIATED ORGANIC COMPOUNDS

Diesel exhaust is inhaled as a complex physicochemical mixture of organic aerosols, vapor-phase organic and inorganic compounds, and particle-adsorbed materials. The particles and their composite materials distribute along the bronchoalveolar tract according to size, as discussed above. The polar and water-soluble gases tend to be removed in the nasal passages and the proximal bronchi. Reactive oxidants interact maximally at the bronchoalveolar junction. The organic compounds may be desorbed by the lipids of the alveolar lining layer and possibly into the intracellular fluids of the phagocytic cells. As the phagocytizing macrophages move through the lungs, the dosage of the different chemicals may be delivered at different points along the respiratory tract and at different rates to the bronchoalveolar fluids, the underlying cells, and the lymphatic and blood circulations. The extent to which organic compounds desorb from the particles determines their bioavailability for subsequent reactions with cellular molecules such as DNA.

The critical factors in the bioavailability of the active mutagenic polycyclic aromatic hydrocarbons (PAHs)* of the diesel particle are (1) the surface structure of the particle, (2) the composition of the adsorbed organic compounds, (3) the composition of the extracellular and intracellular fluids, (4) the balance of the molecular binding forces between the particle and the adsorbed organic molecules, on the one hand, and the extracting biologic fluids on the other, and (5) the metabolism of the desorbed chemical. The physicochemical properties of the vapor-particle linkage, such as molecular binding energies, probably determine the bioavailability of the organic chemical at the site of deposition of diesel exhaust particles in the bronchioles and alveoli (Gerde et al. 1991).

The structure of the diesel exhaust particle is described in the background paper by Sawyer and Johnson in this report. Briefly, the particle is a chain and cluster of carbonaceous aggregates, with PAHs adsorbed on the surfaces of the interstices. Adsorbed organic compounds may not be readily bioavailable because of high binding energies, which prevent the release of the compounds to the cell. In general, organic vapor molecules occupy the tightest binding sites

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

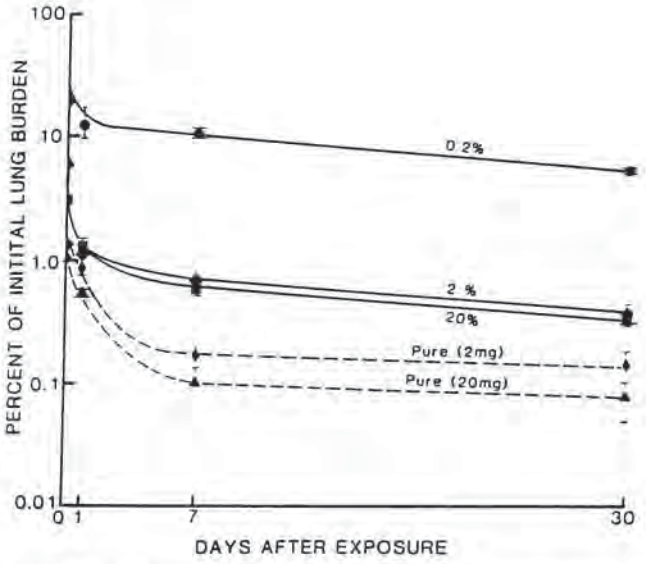


**Figure 5. Lung clearance of $^{14}C$ following nose-only inhalation exposure of rats to aerosols of pure [$^{14}C$]BaP (2 or 20 mg/m$^3$) or [$^{14}C$]BaP adsorbed onto carbon black particles at 0.2%, 2.0%, or 20% by mass** (total particle concentration = 79–100 mg/m$^3$). As a function of time, retention values are expressed as percentages of the calculated initial deposition of $^{14}C$ in lungs based on a deposition value of 15% and a rate per minute volume of 0.26 L/min. Values are means ± SE. (From Sun JD, Wolff RK, Maio SM, Barr EB, 1989, Inhalation Toxicol 1[1]:10, Taylor and Francis, Inc., Washington, DC. Reproduced with permission. All rights reserved.)

(greatest binding energies) on the particle first, and then remaining molecules occupy sites with lower binding energies. Thus, when adsorbed onto particles at lower concentrations, organic compounds are more tightly bound than at higher concentrations. The studies of Sun and coworkers (1989) (Figure 5) support this point, showing much longer retention of benzo[*a*]pyrene (B*a*P) adsorbed onto carbon particles at low (0.2%) versus high (2.0% or 20%) concentrations. In contrast, particles consisting entirely of PAHs (Ebert 1990) may be far more bioavailable because no solid carbon core exists to exert binding energy and inhibit dissolution of the organic compounds into the lipid layers. The physicochemical behavior of the gas-particle relation is the critical factor in bioavailability.

An additional factor in the rate of release of potentially bioactive compounds is the degree of agglomeration of free and intracellular particles (Gerde et al. 1991), which occurs with tracheal instillation (Sun et al. 1989) and with inhalation at the high exposure levels used to produce lung tumors in animals. Agglomeration appears to retard the release of the organic compounds and to prolong the dose administration to the lung cells.

Compared with aerosols of pure organic compounds, adsorption of mutagenic organic compounds to diesel exhaust particles increases their deposition in the lungs and prolongs their retention and the time-course of their release (Creasia et al. 1976; Sun et al. 1983, 1984, 1989; Sun and McClellan 1984; Bond et al. 1986; Wolff et al. 1989) (Figure 6). Adsorption of volatile organic compounds to particles also increases postexposure covalent binding of the organic molecule to lung macromolecules (Figure 7).

The thickness of the respiratory tract fluid layer may be a significant factor in the rate at which chemicals are delivered to the underlying epithelium in the larger airways. The presence of lipid surfactant material in the alveoli, however, may delay the penetration of lipophilic deposits and allow surface flows to remove the chemicals before the penetration of material to underlying cells (Gerde and Scholander 1989).

Finally, the intracellular environment has a mitigating effect on the bioavailability and toxicity of adsorbed organic compounds. Phagocytosis of diesel particles by alveolar macrophages sharply reduces the mutagenicity of particles subsequently released from the cells (King et al. 1983; Bond et al. 1984). Phagocytosis also diminishes the quantity of extractable organic compounds, presumably by intracellular metabolism of the organic compounds. The mutagenicity of these organic compounds in vivo is significantly less than predicted from mutagenicity studies of chemically extracted material (Brooks et al. 1981; King et al. 1981; summarized by McClellan et al. 1982 and by Vostal 1983) (Figure 8). These data clearly show that extraction of particle-adsorbed organic compounds by relevant lung-based fluids is far less efficient than by chemical solvents (King et al. 1981). Furthermore, organic compounds extracted by biologic fluids simulating surfactant and respiratory tract fluids are dramatically less mutagenic in bacterial mu-

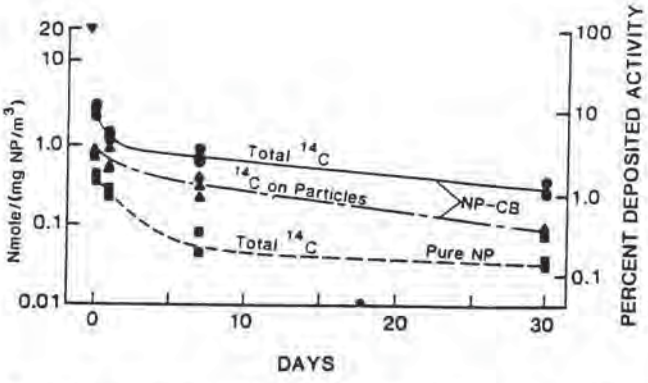


**Figure 6. Lung retention of $^{14}C$ plotted as nitropyrene (NP) equivalents per milligram NP per cubic meter.** Total $^{14}C$ in lung (●) and $^{14}C$ bound to particles (▲) are shown for rats exposed to NP adsorbed to carbon black particles. Total $^{14}C$ in lung (■) is shown for rats exposed to pure nitropyrene. Estimated initial deposited activity (▼) is shown for reference, and the axis on the right shows a scale calculated as the percentage of estimated initially deposited activity. The curves shown were calculated using best-fit two-component exponential functions. (From Wolff RK, Sun JD, Barr EB, Rothenberg SI, Yeh HC, 1989, J Toxicol Environ Health 26:318, Taylor and Francis, Inc., Washington, DC. Reproduced with permission. All rights reserved.)

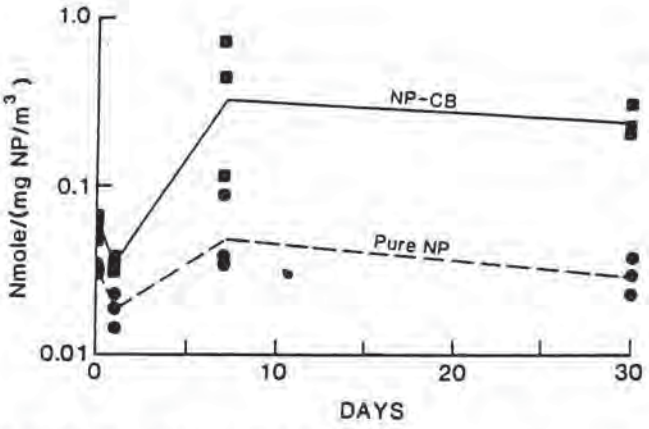


**Figure 7. Activities of $^{14}C$ covalently bound to lung tissue plotted as nitropyrene (NP) equivalents per milligram NP per cubic meter for rats exposed to NP adsorbed to carbon black particles (■) and for rats exposed to pure NP aerosols (●).** The lines shown join the means of the values at 7 days and 30 days (insufficient data to allow best-fit functions). (From Wolff RK, Sun JD, Barr EB, Rothenberg SJ, Yeh HC, 1989, J Toxicol Environ Health 26:319, Taylor and Francis, Inc., Washington, DC. Reproduced with permission. All rights reserved.)

tagenicity test systems (Siak et al. 1980). These findings tend to diminish the role of particle-adsorbed mutagens in the pathogenesis of diesel-associated lung cancer.

In summary, adsorption of organic molecules to carbonaceous particles enhances their penetration into the respiratory portions of the lungs but diminishes their bioavailability in proportion to the binding energy of the organic molecules and the agglomeration of the particle. Organic compounds may be metabolized on the particle surface or after release, and may follow the particle in its pathway of clearance from the lungs. The effect of the biologic environment is to reduce the bioavailability of the particle-adsorbed organic compounds. The extracellular and intracellular environments are less able than chemical solvents to extract the organic compounds from the particle (probably because of weak nonpolar bonding), and the released organic compounds are less mutagenic than chemical extracts in bioassays.

## RELATION OF PARTICLE LOCATION TO THE TOXICOLOGIC RESPONSE

One of the important factors in determining exposure-dose relationships to particle inhalation in the lung is the "moving target" characteristic of the migrating, particle-laden macrophage. If the particle dose is directly responsible for the subsequent pathology, then it is reasonable to expect a physical proximity of the particles and the pathology. If, however, the particle initiates a cascade of events that only distantly induces pathology, then geographic proximity is not necessary.

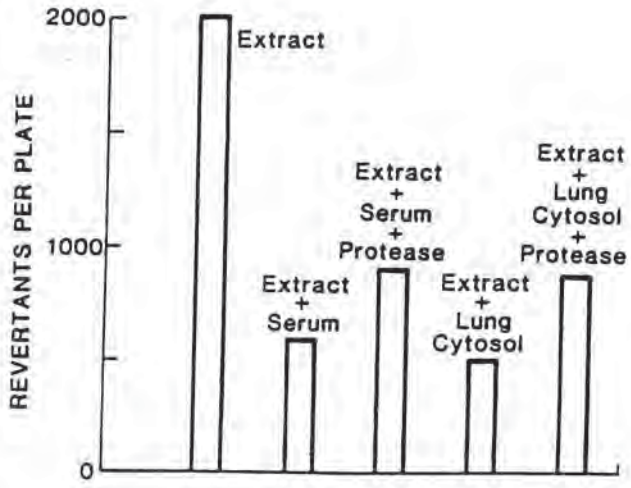


**Figure 8. Influence of serum and lung cytosol on the mutagenicity of diesel exhaust particle extracts.** (Reprinted with permission from Elsevier Service Publishing Co. and R.P. McClellan, as published in McClellan et al. 1982.)

Literature on the dynamic nature of particle movement, relative dose, and resultant toxicologic effect is particularly limited. After deposition and ingestion by macrophages, particles are variously transported either as free particles or within alveolar macrophages to the alveolar ducts and terminal bronchioles on their way to the mucociliary epithelia of the bronchioles; alternatively, particles may reach the local and regional lymphatics and be transported to peribronchial, perivascular, and subpleural sites of long-term storage. Although much of the understanding of intrapulmonary deposition and clearance has been obtained with carbon and other inert dusts (e.g., see Sorokin and Brain 1974), recent studies (Mauderly et al. 1986, 1994) indicate that diesel exhaust particles follow similar physiologic distributions. The level and precise cellular location of that dose may be critical to its ability to induce toxicity.

Well-documented studies of lung tumors induced by diesel exhaust have been performed by Mauderly and coworkers (1986, 1994) in rats. The tumors were located only in the peripheral lung, in contrast to the bronchial location of most human lung cancers. Diesel exhaust particles deposit and accumulate in the distal lung, and the associated cellular proliferation, inflammation, and hyperplasia take place at the alveolar and bronchiolar levels. Bond and coinvestigators (1988) showed that although the entire respiratory tract surface received exposure to the diesel exhaust particles, only the nasal turbinates and the peripheral lung, sites where particles are retained, showed an excessive level of DNA adducts. Thus these studies demonstrate a geographically quantitative relationship among concentration or duration of dose, biologic effect (adduct formation), and pathological effect.

## EXPOSURE PARAMETERS AND THE DISTRIBUTION OF DOSE

The encounter between a hazardous agent and the host is termed exposure and has parameters pertaining to both the agent and the host. The encounter has time variables of duration, constancy or intermittency, frequency, and rate of delivery; the agent has properties of concentration and physicochemical state; the host brings structural and functional variables associated with state of activity, age, and disease. The interaction of these variables affects the distribution of effective dose in the lungs—that is, the proportional concentration of the agent on the respiratory membrane, in macrophages or epithelial cells, or at other sensitive sites depending on differences in deposition, transport, or cellular ingestion.

Time variables add to exposure, deposited and absorbed dose, and lung burden roughly in proportion to the total

time of exposure. Little of the available evidence, however, indicates that time variables affect the distribution of dose in the respiratory tract. The concentration of the agent may affect the distribution of dose such that it exceeds the capacity of proximal tissues to absorb, neutralize, or clear the agent as it passes along the respiratory tract, but these effects may also be attributable to limitations in the defense mechanisms of the host.

Particle size, vapor solubility, and reactivity are the most significant physicochemical properties of the agent that determine initial dose distribution along the respiratory tract. The range of sizes for diesel exhaust particles results in the majority of particles depositing in the pulmonary region. As noted above, technological changes in fuel characteristics or engine design may affect particle size and hence regional distribution of particles in the respiratory tract. The studies of Kleinman and Mautz (1991) using formaldehyde and ammonium nitrate particles showed that the addition of ammonium nitrate particles to the aqueous-soluble gaseous pollutant increased the penetration of the formaldehyde to the lower respiratory tract and increased the dose to distal lung tissues. In contrast, Jakab and colleagues (1992) found that carbon particles, which did not adsorb formaldehyde, did not increase penetration of the gas into the lungs. Furthermore, Bond and colleagues (1986) found that the addition of nitropyrene to carbon particles did not significantly alter the distribution of the dose. They also found an increase in retention of the nitropyrene aerosol at low exposure concentrations for reasons not readily explained.

Limited information in the literature indicates that the condition of the host also has a significant bearing on the distribution of the dose in the respiratory tract. The physiologic state of the host, whether resting, exercising, or working, alters the ventilation of the lungs as the respiratory system adjusts to the metabolic needs for oxygen intake and carbon dioxide excretion. It is intuitive that increased ventilation of the lungs would increase the dose and alter the distribution of any foreign materials suspended in the inhaled air, such as diesel exhaust. Studies in animals (Valberg et al. 1982) and in humans (Heyder et al. 1986) have demonstrated that increasing the tidal volume leads to an increase in total lung deposition. The shift from nasal to oral breathing that occurs with exercise, however, does not affect the regional distribution of diesel exhaust–sized particles (Heyder et al. 1986). One might expect that age would affect exposure parameters, because the higher surface:mass ratio in smaller bodies requires higher ventilation values to compensate for their greater heat-loss rates. Curiously, Mauderly and associates (1987) found that lung burdens of diesel exhaust particles, when adjusted for lung weight, were identical in adult and developing rats. The accelerated clearance of the particles, however, which they observed in the younger animals during the months after exposure ceased, should reduce the continuing exposure of the pulmonary tissues to particles and the adsorbed organic compounds and may also explain how lung burdens formed after exposure in developing rats.

In addition to activity level and age, disease also may influence the distribution of particles. Mauderly and associates (1989) showed that experimentally induced emphysema in rats decreased the cumulative burden of diesel exhaust particles in chronic exposure conditions. Others (Laube et al. 1986) have found in humans that obstructive breathing patterns, such as those that occur in emphysema, result in more central deposition of particles because of greater turbulence of the airstream; central deposition results in more rapid mucociliary clearance and hence a lower lung burden. Also, persons with chronic bronchitis or other diseases that alter the thickness of the respiratory tract fluid layer may experience lower doses to the underlying epithelium than persons with healthy lungs.

## MATHEMATICAL MODELS OF DEPOSITION AND CLEARANCE KINETICS

Mathematical models of biologic systems are theoretical constructs of physiologic, biochemical, and molecular relationships based on quantitative measurements of components of those systems. The models are designed to extend understanding beyond actual observations and to provide a predictive basis for subsequent experimental testing. Models are particularly helpful when integrating complex phenomena, such as the interactions between compound mixtures of air pollutants and the host defenses of the respiratory tract.

The purposes of mathematical models of pulmonary deposition, clearance, and retention are (1) to test hypotheses about biologic mechanisms, (2) to predict the dose to target cells and tissues from short- or long-term and high- or low-concentration exposures, and (3) to extrapolate from measurements made in animals to risks to health of measured exposures in humans. To the extent that the models accurately characterize underlying biologic events that are comparable across species, they will accomplish these objectives.

One of the most central uncertainties for assessing the risk of measured environmental exposures to pulmonary tissues is the quantitation of dose to the relevant cellular target. The architecture of the airways aerodynamically filters the inhaled air, and transport systems evacuate de-

posited pollutants from the lungs, but the fraction of pollutants affected by these physiological processes varies with particle size, the solubility of the agent, and the breathing status of the host. Mathematical models quantify these variables and attempt to predict the dose to target tissue in a given set of circumstances of exposure.

## SUMMARY OF MODELS CURRENTLY USED

Some models currently in use address single aspects of pulmonary processes. For example, the model of Yeh and Schum (1980) analyzes the effects of breathing pattern on the quantitative deposition of particles from 0.01 to 10 μm using airway morphometric data obtained from a silicone rubber cast of the human tracheobronchial tree. Their model predicts that the fractional deposition in the deep lung increases quantitatively with mouth versus nasal breathing, decreasing particle size, and increasing tidal volume. Therefore, a vigorously laboring worker exposed to a small-particle pollutant, such as diesel exhaust, would receive a fractionately greater dose from a given exposure than a resting individual. Yu and Xu (1986) extended these predictions to experimental animals and confirmed the effect of tidal volume; they also predicted that mouth versus nasal breathing has little effect on fractional deposition with small-particle aerosols, such as diesel exhaust particles. Additionally, they calculated that deposition was relatively insensitive to the particular lung model used to describe airway architecture. This suggests that interindividual and interspecies variability has relatively little influence on deposition, a conclusion that is consistent with experimental data (see discussions above on deposition and clearance). In contrast, these investigators calculated that human infants would receive twice the deposited dose as adult humans, and that lung deposition efficiency in small animals might be slightly higher than in large animals.

Another important parameter when predicting dose to target is the desorption kinetics of particle-adsorbed PAHs; estimates of this parameter are needed to predict the quantitative dose of PAHs to epithelial cells. The mathematical model developed by Gerde and Scholander characterizes the ambient transfer of gas-phase pollutants to particle surfaces and of particle-adsorbed PAHs to biologic fluids (1989), as well as the penetration of PAHs through the respiratory tract fluid to the underlying epithelium (1987). The model predicts that with the low concentrations of PAHs and the time constants available in the ambient environment, only small amounts of PAH will be adsorbed by particles. In vehicle engines, however, the higher concentrations of PAHs in the combustion chamber and tailpipe result in substantial adsorption to carbon particles. Some of what is adsorbed could be released into lipophilic biologic fluids, such as surfactant. If such release occurs, the solubility of these organic desorbents in the lipid phase might retard their penetration to the underlying epithelial cell layer.

The release of adsorbed organic compounds may not be uniform. The model of Yu and Yoon (1991) includes rates for slow and fast desorption of particle-bound organic compounds. The model of Gerde and coworkers (1991) predicts a prolonged low-dose release of PAHs from aggregates of particles because much of the particle surfaces is not exposed to the lipid desorbents. Such particle aggregation would occur with high air concentrations, which is characteristic of animal studies. At low particle concentrations, however, such as with most human exposures, in which there is immediate and extensive surface contact with respiratory tract fluid layers, rapid point release is predicted. These predictions are supported by experimental data, as shown in Table 6, which is taken from their report.

Another group of models (Soderholm 1982; Yu et al. 1988; Stöber et al. 1989; Strom et al. 1989; Yu and Yoon

**Table 6.** Comparison Between Model Simulations and Existing Experimental Data on Elimination of Particle-Associated Benzo[*a*]pyrene from the Rat Lung[a]

| Experiment | Administered Amount | Mode of Administration | Time for 90% Pulmonary Elimination | Corresponding Diameter of Simulated Aggregate |
|---|---|---|---|---|
| Sun et al. 1982 | ~ 1 μg[b] | Inhalation, crystals on gallium oxide | < 30 min | < 10 μm |
| Henry et al. 1975 | 0.9 mg | Instillation, crystals on iron oxide | 50 hr | 100 μm |
| Schreiber et al. 1975 | 8 mg | Instillation, crystals on iron oxide | 14 d | 250 μm |

[a] Reprinted with permission from Academic Press and P. Gerde, as published in Gerde et al. (1991).

[b] Estimated from the deposition of gallium oxide.

1991) construct an interlocking multicompartmental system that receives a fraction of the inhaled particles as a deposited dose and transfers the dose to another compartment on the path to excretion or storage. The time constant and particle load for each compartment are calculated from actual experimental data from animal studies. Typically, as in the studies by Stöber and associates (1990), the model is validated against further experiments under different particle loading conditions; also, measures of deposition, intercompartmental transfer, and clearance or storage are compared with the predicted values of the model.

## CHARACTERIZING BIOLOGIC PROCESSES

The four integrative models mentioned above (Soderholm 1982; Stöber et al. 1989; Strom et al. 1989; Yu and Yoon 1991) differ in limited but significant fashion with regard to the number of compartments theorized, the interrelationships of the macrophage populations, macrophage turnover, the role of the interstitium, the existence of a permanent storage (sequestering) compartment, and the transfer linkages among the several intrapulmonary compartments. The model developed by Yu and Yoon (1991) eschews the sequestering compartment and attributes all parenchymal clearance to alveolar clearance occurring nonlinearly and as a function of lung burden. The model of Stöber and colleagues (1989) recognizes the age-related decline in ventilation, which diminishes deposition at a given exposure level. Their model also introduces a factor to express an inherent age-related decline in alveolar clearance function that is independent of lung burden.

Based on confirmation of predicted rates with experimental observations, these integrative models characterize the kinetics of deposition, clearance, and retention quite well for individual animal species. Because deposition appears to be a predictable fraction of the inhaled particle load (k × ventilation × airborne concentration) for a given particle size distribution, the initial dose of particles to the whole lung or a subunit may be accurately predicted. However, the demonstrated mitigating effect of the biologic environment on the bioavailability of adsorbed organic compounds and the instability and wide variance of clearance and lung burden across species and among individuals introduce substantial uncertainty when extrapolating from animals to humans.

The greatest limitation of the mathematical models is the lack of understanding about the underlying biologic processes responsible for the transfer of particles to the interstitium, the transfer of organic compounds to macromolecules, the transport of particle-laden macrophages through the alveoli and to the points of storage or excretion, and the basis for the effects of high lung burdens. Lethargic and degenerating macrophages, particle release and rephagocytosis, or particle-blocked pathways of clearance are all plausible mechanical explanations for these observations, but other explanations are imaginable. Of great interest is the high degree of reproducibility of the "threshold" of overload—the point at which clearance begins to fail, somewhere between 0.6 and 0.8 mg of particles per lung in the rat. Discovery of what governs this "threshold" and the determination of the effect of different species on the "threshold" are critical to the assessment of risk and the upper limits of permissible exposure to diesel and other particulate matter.

## APPLICATION OF MODELS TO HUMANS

The ultimate purpose of models of deposition, clearance, and retention of diesel exhaust materials is to predict more accurately the dose to target tissues in human lungs when experimental data are unavailable. Although no experimental data on the deposition, clearance, and retention of diesel exhaust particles in humans are available, a number of investigators have used radiotracer-tagged inorganic particulate aerosols as surrogates of diesel exhaust particles (Stahlhofen et al. 1980; Yeh and Schum 1980; Bailey et al. 1982; Bohning et al. 1982; Yu and Xu 1987a,b) to characterize deposition and clearance kinetics in humans.

Yeh and Schum (1980) showed good correlation between mathematical predictions of particle deposition and experimental data in an anatomically reconstructed model of the respiratory airways (see Figure 2 of the reference). Data from Chan and Lippmann (1980) confirm that fractional deposition in the human respiratory tract is at a minimum at the particle size range of diesel particulate matter (0.1 to 0.5 μm). Furthermore, these predictive models showed close conformity with the experimental data over a particle size range of 0.1 to 5.0 μm. This conformity extends to the subcomponent of alveolar deposition. Thus it appears that the mathematical models reasonably predict the deposition of diesel exhaust particles in humans.

Yu and Xu (1987a,b) developed a mathematical model of the human lung from birth to adulthood. They used the model to predict the effects of changing morphology and function on the deposition and distribution of inhaled diesel particles. They combined available airway morphometric data from animal species, including humans, with the corresponding values for pulmonary ventilation based on body size and surface:mass ratio to calculate the deposition of diesel particles. They found markedly increased deposition in the infant, with a maximal value twice that of the adult in the nonciliated airways at 2 years of age. The probable reason is the changing relationship between number of alveoli, which increases rapidly in the

first four to five years of life, and total lung volume (alveolar size), which increases until approximately age 20. Deposition also is affected by the rate and depth of ventilation, which differs in infants and children. While these conclusions reflect results of models based on human anatomic and physiologic data, they cannot be verified with experimental measurements using exposure of humans to diesel exhaust particles. Nevertheless, they do provide a rational basis for concern regarding increased risk of infants exposed to particulate matter.

In later studies, Yu and Yoon (1991) and Yu and Xu (1987b) focused on modeling lung clearance, which then allowed calculation of retention and lung burden in relation to exposure conditions. Their model predicted that lung clearance declined as continuous exposure concentrations increased from 100 to 1,000 $\mu g/m^3$ and that lung burdens were insufficient to cause reductions of alveolar clearance in adults or children when continuous air concentrations were maintained below 50 $\mu g/m^3$ (Figure 9). Intermittent exposure would increase by an order of magnitude the concentration of particles tolerated without clearance overload. Because human exposure to diesel exhaust under ambient conditions is intermittent and below these concentration levels (see background paper by Watts, this report), it is unlikely to result in lung burdens sufficient to impair clearance, based on this model. The wide range of variation in clearance values between and within species, however, particularly in humans, renders extrapolation quite tenuous for lung burdens and associated health effects at a given exposure level without further experimental confirmation.

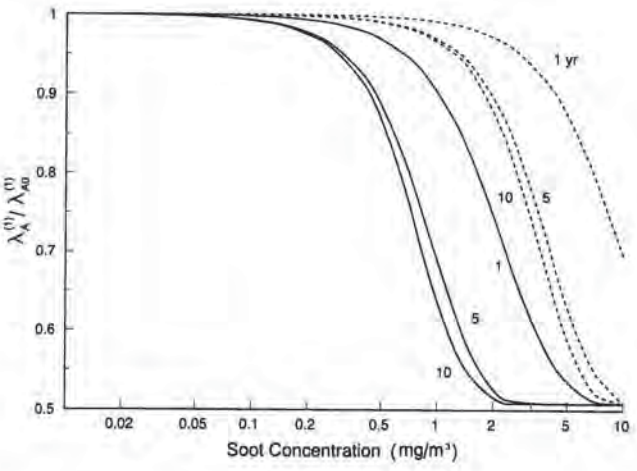


**Figure 9. Normalized clearance rate of diesel soot, $\lambda_A^{(1)}/\lambda_{A0}^{(1)}$ versus soot concentration** in human adults at the end of a continuous exposure of 1, 5, and 10 years. Solid lines represent an exposure pattern of 24 hours/day and 7 days/week and dashed lines represent 8 hours/day and 5 days/week. Reprinted from Yu and Yoon (1991).

## CONCLUSIONS

Six major issues underlie the interpretation of epidemiologic and toxicologic data bearing on the possible health hazards of exposure to diesel exhaust: (1) extrapolation of exposure concentrations measured under experimental conditions to actual dose to target receptor in humans under different conditions of exposure, (2) extrapolation of findings across species from effects in animals to effects in humans, (3) extrapolation of data from acute exposure studies to chronic, (4) extrapolation of data obtained in in vitro studies to intact living organisms, (5) extrapolation of biologic responses found at high dose to projected responses at low dose, (6) extrapolation of mechanisms from one mode of exposure to a different mode. This paper analyzes experimental data relevant to each of these issues and allows the following conclusions to be considered:

***1. Extrapolation of dose under different conditions of exposure.*** Dosimetry is greatly affected by whether exposure at a given concentration is continuous or intermittent, whether at exercise or rest, and whether coexisting factors exist that affect deposition and, especially, clearance. Therefore, extrapolation of dosimetry from data gathered under controlled experimental conditions to that experienced under working or ambient conditions for humans is fraught with uncertainty.

***2. Extrapolation across species.*** Mechanisms of deposition, clearance, and retention are qualitatively similar in different animal species and in humans but differ in their quantitative aspects. Deposition efficiency is quite consistent across species, age, and previous exposure. Clearance rates for inhaled particles, however, show wide quantitative interspecies and intraspecies variability, making extrapolation across species somewhat problematic. Retention, which varies inversely with rate of clearance and may be the quantitative indicator of biologic effect, will differ across species, be greater in previously or simultaneously exposed lungs (possibly above an identifiable lung burden), and be a principal variable in risk assessment considerations. Therefore, dosimetry may be reliably extrapolated qualitatively, but not quantitatively, across species and from animals to humans.

***3. Extrapolation from acute to chronic exposures.*** The alveolar deposition efficiency of fine particles such as those

in diesel exhaust is relatively unaffected by previous exposure. Clearance, however, declines with increasing dose and dose rate above a threshold. Since long-term dosimetry is determined by the differences between deposition and clearance, it can not be reliably extrapolated from acute to chronic exposures.

***4. Extrapolation from in vitro models to in vivo events.*** Mathematical models provide "in vitro" data for dosimetry studies. These integrative models characterize the kinetics of deposition, clearance, and retention quite well for individual animal species and reasonably predict the deposition of diesel exhaust particles in humans. The models predict that lung clearance declines as continuous exposure concentrations rise from 100 to 1,000 $\mu g/m^3$. Intermittent exposure would increase by an order of magnitude the concentration of particles tolerated without clearance overload. Because human exposure to diesel exhaust under ambient conditions is intermittent and below these concentration levels (see the background paper by Watts, this report), it is unlikely to result in lung burdens sufficient to impair clearance, according to this model. The wide range of variation in clearance values between and within species, however, particularly in humans, renders extrapolation quite tenuous for lung burdens and associated health effects at a given exposure level without further experimental confirmation.

***5. Extrapolation of dose.*** In experimental animals, clearance of diesel exhaust particles is maintained at continuous exposure levels in the 50 to 100 $\mu g/m^3$ range. In chronic exposure at high (milligram) concentrations, clearance is retarded by a high dose rate (Bellmann et al. 1983), whereas at low (microgram) levels, clearance is more efficient with high dose rates (Strom et al. 1990). Retention, which varies inversely with rate of clearance, is greater at high-dose than at low-dose rates. In addition, previous lung burden slows lung clearance, and there is a threshold for this effect (600 to 800 $\mu g/g$ of lung tissue in the rat). Thus retention increases with increasing dose and lung burden accumulates more rapidly as clearance fails. Since there appears to be a threshold for this effect, extrapolation of high-dose effects to low dose is unreliable.

***6. Extrapolation of mechanisms.*** Adsorption of organic molecules to carbonaceous particles enhances their penetration into the respiratory portions of the lungs because these molecules follow the deposition and uptake pathways of the particles and end up in macrophages. Once adsorbed onto particles, however, these molecules become less bioavailable because of high-energy binding and the lesser ability of biologic than of chemical fluids to desorb them. Thus mechanisms of carcinogenesis, which are operative in vitro with nonadsorbed mutagenic organic compounds, may not be extrapolated to mechanisms in vivo in which these same compounds are adsorbed to particle surfaces.

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## ABBREVIATIONS

| | |
|---|---|
| B*a*P | benzo[*a*]pyrene |
| $^{14}$C | carbon 14 |
| $^{134}$Cs | cesium–134 |
| $^{134}$Cs-FAP | $^{134}$Cs–fused aluminosilicate particles |
| $^{59}Fe_3O_4$ | iron oxide |
| $^{67}Ga_2O_3$ | gallium oxide |
| PAH | polycyclic aromatic hydrocarbon |
| SD | standard deviation |
| SE | standard error |

# Diesel Emissions and Other Substances Associated with Animal Carcinogenicity

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*Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects*

## INTRODUCTION

Diesel engine emissions are complex mixtures that contain thousands of organic and inorganic substances derived from the complete and incomplete combustion of fuel and lubricating oil (reviewed by Schuetzle and Frazier 1986; International Agency for Research on Cancer 1989a). These substances are emitted partly in the vapor phase and partly in the particulate phase of the exhaust. The physical and chemical properties of organic compounds and particulate matter in diesel exhaust are described elsewhere in this report (see background paper by Sawyer and Johnson). Additional information is available in appropriate references (Nesnow et al. 1982a; International Agency for Research on Cancer 1989a,b).

This paper deals with issues related to the carcinogenicity of diesel emissions in laboratory animals; these considerations include, but are not necessarily limited to, (1) evidence for the carcinogenicity of inhaled exhaust and its components; (2) roles that different emission components may play in any observed carcinogenic activity; and (3) whether diesel emissions contain substances that act as tumor promoters or cocarcinogens.

## CARCINOGENICITY OF WHOLE DIESEL EXHAUST AND RELATED SUBSTANCES IN LABORATORY ANIMALS

### ANIMAL BIOASSAYS

Comprehensive reviews of the literature and additional research data on earlier studies of the effects of chronic inhalation exposure to diesel exhaust in laboratory animals are available (International Agency for Research on Cancer 1990; Mauderly 1992). Tables 1 to 6 present results of selected experiments in which rats, mice, hamsters, and one group of monkeys were exposed to whole or filtered exhaust, carbon black, or other particles. Some of the criteria used for inclusion of experiments in this paper were (1) repeated exposure to diesel exhaust on a weekly basis for at least 24 months, and (2) maximum particle concentrations of approximately 3 $mg/m^3$, or concentrations of other substances approximating this level. These limitations were selected from results of other studies that indicated that adhering to them ensured adequate concentration and duration to test the potential for carcinogenesis. Most, but not all, of the studies reported herein met these criteria. When possible, the tables list lung tumors of both benign and malignant types.

It should be noted here that there is some disagreement among pathologists (see Appendix A) regarding classification of one of the commonly observed lesions, squamous cysts (or squamous tumors), in the lungs of rats exposed to high concentrations of diesel exhaust. As shown in Table 1, both Heinrich and colleagues in 1986 and Mauderly and associates in 1987 reported the lesions as benign tumors; however, in their recent studies Mauderly and associates (1994; Nikula et al. 1995) did not use the term squamous cystic tumor (Table 2), but Heinrich and colleagues (1994b, 1995) continue to include it (Table 3). The Mauderly pathology working group (Mauderly et al. 1994) considered the cystic lesion (see Appendix A) as nonneoplastic; therefore, lesions with this diagnosis were not included in later tables of tumors. This accounts for the disparities among the lung tumor listings in the various tables. Some list squamous cystic tumors, others do not. Furthermore, other pathology working groups in the United States have concluded that squamous cysts are not neoplasms, bringing the matter to a difference of opinion between American and European pathologists. In this background paper the lung tumors and other lesions were categorized as they were reported by the investigators. The data from studies listed in all of the tables are considered in greater detail later in this paper.

Studies reported in this paper confirm that diesel exhaust is a carcinogen for at least two strains of rat (F344 and Wistar) when the particle concentration is equal to or greater than approximately 3 $mg/m^3$ and exposure is seven or more hours per day, five days per week, for 24 months. Whereas a 24-month duration is used for most carcinogenesis studies, lung tumors associated with diesel exhaust occur late, and this duration may need to be extended for future studies. To do so, however, introduces other confounding factors of age. It should be pointed out that at the above noted concentration, or greater, the normal clearance mechanisms of the lung are overloaded (Wolff et al. 1987; Creutzenberg et al. 1990; Morrow 1992). This results in continued, progressive accumulation of particles, which is now accepted by most investigators as significant in the development of lung tumors in this species (Heinrich et al. 1986, 1994b, 1995; Mauderly 1994). A single negative study, presented here for comparative purposes (Ishinishi et al. 1988), used a maximum particle concentration of 2.3 $mg/m^3$. The data also indicate that emissions from the various engines tested were comparable with respect to oncogenic effects.

The progressive accumulation of particles and the effects of these on tumorigenesis have been documented by recent studies using carbon black. The more recent studies reported by Mauderly and associates (1994; Nikula et al. 1995) and Heinrich and colleagues (1994b, 1995) strongly

**Table 1.** Data from Earlier Studies on Lung Tumor Incidence and Types in Rats After Chronic Inhalation of Whole Diesel Exhaust

| Animals | | Exposure Conditions | | | Benign Tumors (%) | | Malignant Tumors (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain (Gender) | Rats per Group | Engine | Time (hr/d × d/wk × mo)[a] | Particle Concentration ($mg/m^3$) | Squamous Cystic Tumor | Adenoma | Adeno-carcinoma | Squamous Cell Carcinoma | Adeno-squamous Carcinoma | Total No. of Tumors | Total Animals with Tumors (%) | Reference |
| F344 (F) | 22 | 2.4-L Truck | 8 × 7 × 24 (6) | 0 | — | 4.5 | — | — | — | 1 | 4.5 | Iwai et al. 1986 |
| | 19 | | | 4.9 | — | 15.8 | 5.3 | 5.3 | 10.5 | 8 | 42.1[b,c] | |
| Wistar (F) | 96 | 1.6-L Volkswagen | 19 × 5 × 32 | 0 | — | — | — | — | — | 0 | 0 | Heinrich et al. 1986 |
| | 95 | | | 4.2 | (6.3–8.4)[d] | (6.3–8.4)[d] | — | 1.1 | — | 17 | 15.8[c] | |
| F344 (M+F) | 220 | 5.7-L Oldsmobile | 7 × 5 × 30 | 0 | — | — | 0.9 | — | — | 2 | 0.9 | Mauderly et al. 1987 |
| | 220 | | | 0.35 | — | — | 1.3 | — | — | 3 | 1.3 | |
| | 220 | | | 3.5 | 0.9 | 2.3 | 0.5 | — | — | 8 | 3.6[c] | |
| | 220 | | | 7.1 | 4.9 | 0.4 | 6.6 | 0.9 | — | 33 | 12.8[c] | |
| F344 (M+F) | 123 | 1.8-L | 16 × 6 × 30 | 0 | — | 0.8 | — | 0.8 | 1.6 | 4 | 3.3 | Ishinishi et al. 1988 |
| | 123 | | | 0.1 | — | 0.8 | 0.8 | 0.8 | — | 3 | 2.4 | |
| | 125 | | | 0.4 | — | 0.8 | — | — | — | 1 | 0.8 | |
| | 123 | | | 1.1 | — | — | 4.1 | — | — | 5 | 4.1 | |
| | 124 | | | 2.3 | — | 0.8 | 0.8 | — | 0.8 | 3 | 2.4 | |
| | 123 | 11-L | 16 × 6 × 30 | 0 | — | — | 0.8 | — | — | 1 | 0.8 | Ishinishi et al. 1988 |
| | 123 | | | 0.5 | — | — | — | 0.8 | — | 1 | 0.8 | |
| | 125 | | | 1.0 | — | — | — | — | — | 0 | 0 | |
| | 123 | | | 1.8 | — | — | 2.4 | — | 0.8 | 4 | 3.3 | |
| | 124 | | | 3.7 | — | — | 4.0 | 1.6 | 0.8 | 8 | 6.5[c] | |
| F344 (M+F) | 260 | 1.5-L Volkswagen | 16 × 5 × 24 (6) | 0 | | | | | | | 1.2 | Brightwell et al. 1989 |
| | 143 | | | 0.7 | | | | | | | 0.7 | |
| | 144 | | | 2.2 | | | | | | | 9.7[c] | |
| | 143 | | | 6.6 | — | (38.5)[e] | (18.3)[e] | (33.7)[e] | (8.7)[e] | 104 | 38.5[c] | |

[a] The number of months some of the animals were exposed to clean air after diesel exhaust exposure is in parentheses.

[b] Includes one rat with large-cell carcinoma of the lung.

[c] Significantly different from control ($p < 0.05$); for detailed data on carbon black refer to Tables 2 and 3.

[d] Calculated range of tumor incidence based on possibility of multiple tumors present in one animal.

[e] Proportion of types of lung tumors. Total less than 100% due to an untabulated mesothelioma in one rat.

support a conclusion that solid particles, including carbon black or the carbonaceous core of diesel emission, play a significant role, most likely an etiologic one, in the oncogenic effects of whole diesel engine exhaust reported in laboratory animals (Tables 2, 3, and 4). Moreover, another type of particle, titanium dioxide ($TiO_2$)*, was also carcinogenic in the Wistar rat (Table 4). The results and their implications for diesel emissions carcinogenicity are explored in some detail later in this paper.

Results of inhalation experiments using mice and hamsters as the test animals suggest that these species are not as sensitive to diesel exhaust and other particle exposures as rats (Table 5); in fact, lung tumors have not been reported in hamsters following exposure to diesel exhaust in any of the studies to date (Heinrich et al. 1986; Brightwell et al. 1989). Studies in mice have yielded equivocal results in strains of this species (Heinrich et al. 1986, 1995; Takemoto et al. 1986; Pepelko and Peirano 1983); concentrations of particles up to 7 mg/m$^3$ were largely ineffective even though this level of exposure created lung overload. The data from Heinrich and colleagues (1986) in female NMRI mice may now be interpreted as negative in view of the more recent studies with the same mouse strain at higher concentrations of diesel exhaust (Heinrich et al. 1995).

The results of one study using cynomolgus monkeys (males only) suggested that this species is also resistant to the effects of diesel exhaust or coal dust (Table 5). The duration of the study, however, was only one or two years, a small part of the life span of this species; in addition, the concentrations of coal dust or diesel exhaust (2 mg/m$^3$) and of coal dust plus diesel exhaust (1 mg/m$^3$ each) were inadequate to test the potential carcinogenicity of the two materials. Thus neither the duration of exposure nor the concentrations of coal dust and diesel exhaust were sufficient

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

**Table 2.** Data from More Recent Studies by Mauderly and Coworkers on Lung Tumor Incidence and Types in F344 Rats After Chronic Inhalation of Diesel Exhaust or Carbon Black[a,b]

| Rats per Group | Gender | Air, Diesel Particle[d], or Carbon Black Concentration[e] (mg/m$^3$) | No. of Lung Tumors[c] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Adenoma | Adeno-carcinoma | Squamous Cell Carcinoma | Adeno-squamous Carcinoma | Total No. of Tumors | Total Animals with Tumors (%) |
| 109 | M | Clean air | 1 | 1 | 1 | 0 | 3 | 2.8 |
| 105 | F | Clean air | 0 | 0 | 0 | 0 | 0 | 0 |
| 106 | M | Carbon black (2.5) | 1 | 1 | 0 | 0 | 2 | 1.9 |
| 107 | F | Carbon black (2.5) | 2 | 6 | 0 | 0 | 8 | 7.5 |
| 106 | M | Carbon black (6.5) | 0 | 1 | 2 | 1 | 4 | 3.8 |
| 105 | F | Carbon black (6.5) | 13 | 20 | 1 | 1 | 28 | 26.7 |
| 105 | M | Diesel exhaust (2.5) | 2 | 1 | 2 | 0 | 5 | 4.8 |
| 105 | F | Diesel exhaust (2.5) | 5 | 3 | 1 | 0 | 8 | 7.6 |
| 106 | M | Diesel exhaust (6.5) | 4 | 3 | 2 | 0 | 9 | 8.5 |
| 106 | F | Diesel exhaust (6.5) | 19 | 19 | 1 | 1 | 29 | 27.4 |

[a] Data are from Mauderly et al. (1994) and Nikula et al. (1995).

[b] Several individual rats had multiple types of tumors and/or multiple tumors of a single type; thus these rats were counted more than once in this table.

[c] Includes all rats killed or dying spontaneously that received gross necropsy and microscopic examinations of the lung (significance not shown in this table).

[d] Diesel exhaust from 1988 6.2-L General Motors engines.

[e] Animals were exposed for 16 hours/day and 5 days/week for 24 months.

**Table 3.** Data from More Recent Studies by Heinrich and Coworkers on Lung Tumor Incidence and Types in Female Wistar Rats Exposed to Diesel Exhaust[a] or Irritant Gases, Carbon Black, and Tar/Pitch Aerosol[b]

| Exposure Conditions | | | No. of Lung Tumors | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Rats per Group | Time (hr/d × d/wk × mo)[c] | Substance (Concentration) | Squamous Tumor | Adenoma | Adeno-carcinoma | Squamous Cell Carcinoma | Adeno-squamous Carcinoma | Total No. of Tumors[d] | Total Animals with Tumors (%) |
| 217 | 18 × 5 × 24 | Clean air | 0 | 0 | 1 | 0 | — | 1 | 0.5 |
| 198 | 18 × 5 × 24 | Diesel exhaust (0.8 mg/m$^3$) | 0 | 0 | 0 | 0 | — | 0 | 0 |
| 200 | 18 × 5 × 24 | Diesel exhaust (2.5 mg/m$^3$) | 7 | 2 | 1 | 0 | — | 11[e] (4) | 6 (2) |
| 100 | 18 × 5 × 24 | Diesel exhaust (7.0 mg/m$^3$) | 14 | 4 | 5 | 2 | — | 29[f] (15) | 22 (9) |
| 72 | 17 × 5 × 10 | Irritant gases[g] | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| 72 | 17 × 5 × 20 | Irritant gases | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 72 | 17 × 5 × 10 | Carbon black (6 mg/m$^3$) | 7 | 2 | 4 | 1 | 0 | 14 | 18 |
| 72 | 17 × 5 × 20 | Carbon black (6 mg/m$^3$) | 4 | 1 | 0 | 1 | 0 | 6 | 8 |
| 72 | 17 × 5 × 10 | Tar/pitch 20[h] | 0 | 0 | 2 | 1 | 0 | 3 | 4 |
| 72 | 17 × 5 × 20 | Tar/pitch 20 | 4 | 0 | 0 | 20 | 0 | 24 | 33 |
| 72 | 17 × 5 × 10 | Tar/pitch 50 | 0 | 0 | 0 | 28 | 0 | 28 | 39 |
| 72 | 17 × 5 × 20 | Tar/pitch 50 | 0 | 1 | 1 | 68 | 0 | 70 | 97 |
| 72 | 17 × 5 × 10 | Carbon black (2 mg/m$^3$)+ tar/pitch 50 | 0 | 0 | 1 | 64 | 0 | 65 | 89 |
| 72 | 17 × 5 × 20 | Carbon black (2 mg/m$^3$)+ tar/pitch 50 | 0 | 0 | 0 | 69 | 0 | 69 | 96 |
| 72 | 17 × 5 × 10 | Carbon black (6 mg/m$^3$)+ tar/pitch 50 | 0 | 4 | 1 | 44 | 1 | 50 | 72 |
| 72 | 17 × 5 × 20 | Carbon black (6 mg/m$^3$)+ tar/pitch 50 | 0 | 0 | 3 | 68 | 2 | 73 | 96 |
| 72 | 17 × 5 × 10 | Irritant gases+ tar/pitch 50 | 0 | 0 | 0 | 6 | 0 | 6 | 8 |
| 72 | 17 × 5 × 20 | Irritant gases+ tar/pitch 50 | 0 | 1 | 6 | 53 | 1 | 61 | 81 |

[a] Heinrich et al. (1995).
[b] Heinrich et al. (1994b).
[c] After exposure for 10, 20, or 24 months, animals were housed in clean air for 20, 10, and 6 months, respectively.
[d] Data in parentheses omit squamous tumors.
[e] One animal had a bronchiolar papilloma.
[f] One animal had a hemangioma.
[g] Irritant gases = 5 ppm nitrogen oxide + 5 ppm sulfur dioxide + 3 ppm formaldehyde.
[h] Hard coal tar/pitch condensation aerosol containing either 20 μg/m$^3$ BaP in 1.1 mg/m$^3$ of aerosol (tar/pitch 20) or 50 μg/m$^3$ BaP in 2.6 mg/m$^3$ of aerosol (tar/pitch 50).

to evaluate carcinogenicity, even if the species were susceptible to the oncogenic effects of either of these substances (coal dust or diesel exhaust, or a mix of the two).

Tables 1 through 5 list tumor incidences and types for a number of studies; these data confirmed that several types of lung neoplasms were induced by diesel emissions, carbon black, and another type of particle ($TiO_2$). With one exception (a hemangioma), all tumors were of epithelial origin, including both benign and malignant types. Table 6 lists the chronology of lesions leading up to the final endpoint of tumors, as described by Mauderly and associates (1994; Nikula et al. 1995). The carefully documented results of this study confirm that there are significant chronological events relative to the pathogenesis of lung tumors associated with diesel exhaust and its components, as has been pointed out by others (e.g., Ishinishi et al. 1986, 1988). Current accepted descriptions and interpretations of lung lesions, including neoplasms, are addressed in the following section.

## LUNG TUMORS IN RODENTS EXPOSED TO DIESEL EXHAUST AND CARBON BLACK

### A Description of Neoplasms

A description of the categories of tumors observed in the experimental animals used in these studies and their probable biological significance is presented before proceeding to a discussion of the lung tumors reported in this background paper. A detailed discussion is provided in Appendix A and in the literature (Jones et al. 1985; International Agency for Research on Cancer 1990).

In the classic sense the term "tumor" has been used for centuries to denote a swelling; for example, the presence of an easily defined mass of tissue such as a scar, a granuloma, an abscess, or a parasitic nodule fulfills the generic definition. In the minds of the average citizen, however, and in actual practice, a tumor is equated with cancer. A tumor does indeed imply a new growth, commonly referred to by pathologists as a neoplasm. Neoplasms are categorized into two major classes—benign or malignant. A tumor is said to

**Table 4.** Effects on Lung Tumor Incidence and Type in Female Wistar Rats Exposed to Diesel Exhaust, Carbon Black, or Titanium Dioxide for Two Years and Held for an Additional Six Months[a]

| | Exposure Conditions | | Lung Load | | No. of Lung Tumors | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Rats per Group | Time (hr/d × d/wk × mo) | Substance (Concentration) | 24 Months (mg/lung) | Cumulative Exposure ($g/m^3 \cdot hr$) | Squamous Tumors | Adenoma | Adeno-carcinoma | Squamous Cell Carcinoma | Total Animals with Tumors (%) |
| 217 | Continuous | Clean air | 0 | 0 | 0 | 0 | 1 | 0 | 0.5 |
| 100 | 18 × 5 × 24 | Diesel particles (7.5 $mg/m^3$) | 64 | 62 | 13 | 4 | 5 | 3 | 22.0 |
| 100 | 18 × 5 × 24 | Carbon black (Printex 90) (7.5 $mg/m^3$ for 4 mo, then 12 $mg/m^3$) | 44 | 102 | 20 | 13 | 13 | 4 | 39.0 |
| 100 | 18 × 5 × 24 | Titanium dioxide P25 (7.5 $mg/m^3$ for 4 mo, 15 $mg/m^3$ for 4 mo, then 10 $mg/m^3$) | 39 | 88 | 20 | 4 | 13 | 2 | 32.0 |

[a] Data are from Heinrich et al. (1992, 1994b, 1995); and Heinrich (1994).

**Table 5.** Long-Term Inhalation Studies of Diesel Exhaust with Species Other Than the Rat

| Animals | | Exposure Conditions | | | Lung Tumors (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strain (Gender) | No. per Group | Engine Size | Time (hr/d × d/wk × mo) | Particle Concentration ($mg/m^3$) | Adenoma | Adeno-carcinoma | Squamous Cell Carcinoma | Adeno-squamous Carcinoma | Total Animals With Tumors (%) | Reference |
| NMRI mice (F) | 84<br>76 | 1.6-L Volkswagen | 19 × 5 × 26 | 0 (Clean air)<br>4.0 | 11<br>15 | 2.4<br>17 | 0<br>0 | 0<br>0 | 13<br>32 | Heinrich et al. 1986 |
| NMRI mice (F) | 80<br>80 | 1.6-L Volkswagen | 18 × 5 × 13.5[a] | 0 (Clean air)<br>7.0 | 25<br>21.8 | 15.4<br>15.4 | 0<br>0 | 0<br>0 | 30[b]<br>32.1[b] | Heinrich et al. 1995 |
| ICR mice (M + F) | 60<br>56 | 269-cc Yanmar | 4 × 4 × 13–28 | 0 (Clean air)<br>2.0–4.0 | 10<br>17.9 | 1.7<br>7.1 | 0<br>0 | 0<br>0 | 12<br>25 | Takemoto et al. 1986 |
| C57BL/6N mice (M + F) | 51<br>150 | 269-cc Yanmar | 4 × 4 × 13–28 | 0 (Clean air)<br>2.0–4.0 | 2.0<br>8.0 | 0<br>3.3 | 0<br>0 | 0<br>0 | 2<br>11 | Takemoto et al. 1986 |
| C57BL/6N mice (F) | 120<br>120 | 1.6-L Volkswagen | 18 × 5 × 24[c] | 0 (Clean air)<br>4.5 | | | | | 5.1[d]<br>8.5[d] | Heinrich et al. 1995 |
| SENCAR mice (M)[e] | 105<br>101 | 3.24-L Nisssan | 8 × 7[f] | 0 (Clean air)<br>6.0–12.0[g] | 3.8<br>4.0 | 0.0[g]<br>2.0[g] | | | 3.8<br>5.9 | Pepelko and Peirano 1983 |
| SENCAR mice (F)[e] | 111<br>104 | 3.24-L Nissan | 8 × 7[f] | 0 (Clean air)<br>6.0–12.0[g] | 6.3<br>16.3 | 0.9[g]<br>0.0[g] | | | 7.2<br>16.3 | Pepelko and Peirano 1983 |
| Syrian golden hamsters (M + F) | 48<br>96 | 1.6-L Volkswagen | 19 × 5 × 28 | 0 (Clean air)<br>4.2 | | | | | NA[i]<br>NA | Heinrich et al. 1986 |
| Syrian golden hamsters (M + F) | 410<br>203 | 1.6-L Volkswagen | 16 × 5 × 24 | 0 (Clean air)<br>6.6 | | | | | NA<br>NA | Brightwell et al. 1989 |
| Cynomolgus monkeys (M) | 15<br>15<br>15<br>15 | 7.0-L Caterpillar | 7 × 5 × 24 | 0 (Clean air)<br>2.0 (Coal dust)<br>2.0 (Diesel particles)<br>1.0 (Diesel particles) + 1.0 (Coal dust) | | | | | NA<br>NA<br>NA<br>NA | Lewis et al. 1986, 1989a |

[a] Exposure was followed by clean air for up to 9.5 months.

[b] Total animals with tumors is less than the sum of individual tumor types because some animals had more than one type of tumor.

[c] Exposure was followed by clean air for up to 6 months.

[d] Individual tumor types were not specified.

[e] Data were taken from Pepelko and Peirano 1983, Table 4-5, p. 278, for clean air or diesel exhaust only.

[f] Exposure began at weaning of parents and continued through mating, pregnancy, and parturition. Exposure of offspring continued from weaning to 15 months of age.

[g] Unspecified carcinomas.

[h] Initial diesel exhaust particle concentration was 6 $mg/m^3$ and was increased to 12 $mg/m^3$ when the offspring were 12 weeks old.

[i] NA = not available. No numbers were given but it was stated that there was no difference in tumor incidence among groups.

be benign when its gross and cytologic characteristics are associated with a growth that will remain localized to its site of origin (not spread to other sites) and that has a mass that is generally amenable to surgical removal. Some space-occupying benign tumors, however, depending on size and location, can disrupt the homeostasis of an organ simply by mechanical interference with the function of organs and tissues; therefore, not all benign tumors can be considered more or less harmless.

Malignant tumors are collectively categorized as cancers, a generic term implying that cells of the tumor not only invade locally but can spread to other sites and set up colonies (metastases) that continue to grow in a manner similar to the parent cancer; more significant, these colonies will ultimately result in death of the host unless removed or destroyed. In the words of one expert (Nuland 1994) commenting on how cancer cells operate, "In the community of living tissues the uncontrolled mob of misfits that is cancer behaves like a gang of perpetually wilding adolescents. They are the juvenile delinquents of cellular society." This is an apt description of malignant cells and their behavior.

Diagnosis of tumor type and category is done by a pathologist trained and experienced in the histologic appearance and biologic behavior of neoplasms. Veterinary and experimental pathology are relatively young medical specialties and have relied to a considerable extent on the experience of medical pathologists working from a rich historical background knowledge about the histology, pathogenesis, and behavior of various human tumor types. This knowledge has accrued over several centuries, often with the advantage of extensive biopsies and follow-up. Thus, in studies reported here, the nomenclature for tumor type and biological behavior is similar to that for human tumors. The tumor types in both humans and experimental animals, with a few exceptions, do tend to be quite similar in their histologic appearance and in their biological behavior. However, because of the relatively short experience with rodent tumors (three to five decades) and the markedly shorter life span, and in the interest of human safety, regulatory bodies in general and some pathologists have assumed a conservative position with respect to extrapolating animal tumor data to human risk. For example, substances that cause papillomas or adenomas (which are benign) might be referred to as carcinogens even if they do not produce carcinomas or adenocarcinomas (which are malignant), and when there is little or no evidence that the tumor destroys its host or even shortens its life span.

### Use of Rodent Tumor Data in Estimating Human Health Risk

The conservative stance by public health authorities, as noted above, reflects a level of uncertainty in the minds of those responsible for interpreting and implementing public

**Table 6.** Chronological Order of Lung Lesion Appearance in Rats Following Exposure to Diesel Exhaust or Carbon Black for Varying Periods of Time[a]

| Lesions | Time of Observation (months)[b] | | | | |
|---|---|---|---|---|---|
| | 3 | 6 | 12 | 18 | ≥ 23 |
| Macrophage hyperplasia | + | + | + | + | + |
| Alveolar epithelial cell hyperplasia | + | + | + | + | + |
| Chronic active inflammation | + | + | + | + | + |
| Septal fibrosis | 0 | + | + | + | + |
| Alveolar proteinosis | 0 | + | + | + | + |
| Bronchiolar-alveolar metaplasia (alveolar bronchiolization) | 0 | 0 | + | + | + |
| Interstitial aggregation of particle-containing macrophages | 0 | 0 | 0 | + | + |
| Focal fibrosis | 0 | 0 | 0 | 0 | + |
| Squamous metaplasia | 0 | 0 | 0 | 0 | + |
| Squamous cysts | 0 | 0 | 0 | 0 | + |
| Tumors | 0 | 0 | 1st tumor at 15 months | | + |

[a] Data are from Mauderly et al. (1994).

[b] + = lesion present; 0 = lesion absent.

health policy, based on results of rodent studies such as those reported in this paper. The practice of combining the numbers of adenomas and carcinomas to indicate total tumors observed further reflects the uncertainty and perceived need to assume a conservative position in the use of animal data. There is some disagreement among veterinary pathologists regarding the validity of these assumptions, but in general most would accept the position regarding the combining of adenomas and carcinomas in the same organ. This is based on the logical assumption that if they derive from the same cell type, and both benign and malignant tumors are present, there may be progression from benign to malignant forms. This is the case in particular with liver tumors, which are common spontaneous neoplasms in rodents; it is much less so with lung tumors because they are not common in untreated rodents, except in a few inbred strains of mice (e.g., strain A).

For more detailed descriptions and explanations regarding the nomenclature of tumors and associated lesions, see Appendix A and references.

**Tumors in Rats**

Review of the available data from published reports issued between 1986 and 1993 confirms that all of the investigators observed a similar spectrum of tumors in the lungs of rats exposed to whole diesel exhaust (Table 1), carbon black, or $TiO_2$ (Tables 2, 3, and 4). The spectrum of tumor types included adenomas, adenocarcinomas, squamous cell carcinomas, adenosquamous carcinomas, and a lesion about which there is some disagreement, a "squamous cyst" or "squamous tumor." As noted above, the latter is classified as a benign tumor by some pathologists; others categorize the lesion as a simple squamous cyst (a nontumor). These various tumor types have been particularly well described by Boorman (1985), Mohr and associates (1990), and Mauderly and colleagues (1994; Nikula et al. 1995) (see Appendix A for the latter).

In practice, there appears to be a continuum of stages through which tissues must pass prior to the final development of a malignant tumor. Therefore, a decision to classify a specific lesion into one category or another reflects the subjective opinion of the pathologist based on experience. For the studies presented in Table 1, the investigators did not examine the statistical significance of the incidence of each of the benign and malignant tumor types observed. This was due in part to the small numbers of individual tumor types found in most studies and in part to the uncertainty inherent in classification of animal tumors.

All of the tumors reported from diesel exhaust and related exposures are of epithelial origin, including the squamous types; the latter are presumed to derive through metaplasia (change) of cuboidal epithelium at the bronchiolar-alveolar junction to a squamous type. Furthermore, the tumors were presumed to arise from areas of the lung in which a proliferation of cells had occurred near the junction of the bronchiolar-alveolar epithelium, accompanied by swelling (hypertrophy) of the type II cells of the alveolar epithelium (Ishinishi et al. 1988).

Squamous metaplasia, often found in conjunction with adenomatous hyperplasia, is believed by some investigators to progress to another type of benign lesion (squamous cyst or squamous cystic tumor), and finally, according to this school of thought, the malignant form (squamous cell carcinoma) is observed as an endpoint. This sequence of events in the pathogenesis is subject to some debate among pathologists. Nearly all of the rats exposed to diesel exhaust have exhibited adenomatous hyperplasia, whereas squamous metaplasia was recognized in only a portion of the animals in some studies (Heinrich et al. 1986). The incidence of hyperplasia was proportional to the length of exposure and to particle concentration (Ishinishi et al. 1988).

With some exceptions (Brightwell et al. 1989; Heinrich et al. 1994b, 1995), substantially fewer squamous tumors than adenomatous tumors were observed in the rat inhalation experiments (Heinrich et al. 1986; Mauderly et al. 1987, 1994; Ishinishi et al. 1988; Nikula et al. 1995). An interesting difference between the observations of Mauderly and associates (1994; Nikula et al. 1995) and Heinrich and colleagues (1994b, 1995) was the higher incidence of squamous cell carcinomas in the Wistar rat used in the study of Heinrich and colleagues (Tables 2 and 3) compared with the F344 rat used by Mauderly and associates (1994; Nikula et al. 1995). An important feature of these studies was the late appearance of lung tumors. Mauderly and associates (1987) noted that less than 20% of the tumors occurred in animals at or before 24 months of exposure to diesel exhaust, which is similar to findings reported by Brightwell and coworkers (1989). The more recent studies of Heinrich and colleagues (1994b, 1995) and Mauderly and associates (1994; Nikula et al. 1995) also reported late appearances of tumors.

Prior to the recent reports of Mauderly and associates (1994; Nikula et al. 1995), a logical presumption might have been that the search for neoplasms prior to the 24-month termination point was inadequate; had the investigators looked for tumors at an earlier time, they would have found them. This argument would appear to be refuted by the chronological examination of groups of animals prior to 24 months by Mauderly and associates (Table 6). Only one tumor-bearing rat was found prior to 23 months, and this one was identified at 15 months into the study. Thus, when tumors were looked for at earlier periods, they were not found. Others have reported this result (e.g., Ishinishi et al. 1986, 1988).

The 24-month duration for rat studies is standard protocol for the National Toxicology Program (NTP) and is accepted by regulatory agencies for safety evaluations of most drugs and other chemicals. It would then appear that the 24-month exposure to diesel exhaust and related materials is sufficient for an adequate test. Other questions are raised by exposure beyond 24 months, which is a major portion of the life span of a rat. It is difficult to factor in the influence of age on tumorigenicity, but this is less likely to be a factor when the respiratory system is the target, because lung tumors occurring spontaneously are rare in laboratory rats (Boorman 1985).

Mauderly and coworkers (1994; Nikula et al. 1995) used 10 groups of adequate size (approximately 115 animals each) and performed serial killings and lung clearance analysis. Data in Table 6 reveal some interesting aspects of lung tumor development in the rat with respect to the sequential or chronological order (pathogenesis) of cellular changes leading toward a neoplasm. The carefully documented chronological appearance of the lesions listed in Table 6 may help to identify important sequential histologic changes leading to tumor induction. First, with one exception, tumors developed only beyond 18 months in this study. Second, the first tumor-bearing rat exhibited lesions characteristic of the increased severity and incidence of bronchiolar-alveolar metaplasia and interstitial aggregation of particle-containing macrophages; accumulation of particle-containing macrophages appears to be a significant aspect of tumor induction. Third, the late appearance of tumors coincided with bronchiolar-alveolar metaplasia, aggregation of particle-containing macrophages in the interstitium, and focal fibrosis. In the focal lesions, an accumulation of debris composed to a significant degree of dead macrophages was observed; these cells had disintegrated and released particulate matter, permitting more extensive exposure of local tissues to effects of the particles, as well as to the degradation products from the disintegrating macrophages (e.g., enzymes).

### Tumors in Mice

The studies with both sexes and four strains of mice, including the SENCAR strain, indicated an equivocal susceptibility to diesel exhaust carcinogenicity. Table 5 lists results of the various trials, showing the relatively small increases in incidences of adenomas and adenocarcinomas in exposed mice. No squamous tumors were observed in any of the mice, but adenomatous hyperplasia was apparently present in a majority of them, even in untreated animals.

Increases in lung tumors in SENCAR mice were reported after 15 months (Table 5) of postnatal exposure to diesel exhaust (Pepelko and Peirano 1983). This study conducted by the U.S. Environmental Protection Agency (EPA) was interesting because exposure began with the parent generation and continued through pregnancy and lactation to weaning.

### Tumors in Species Other Than Rats and Mice

Investigations to date using hamsters (Heinrich et al. 1986; Brightwell et al. 1989) have not demonstrated a carcinogenic effect associated with diesel exhaust in this species when the exposure concentration was either 4.0 or 6.6 $mg/m^3$ for 24 or 28 months (Table 5). Hamsters are considered to be a useful species when studying respiratory disease because of their relative lack of indigenous secondary infections compared with the rat (Saffiotti 1969). They have been used widely for lung carcinogenesis investigations but have been nonresponsive to diesel exhaust, confirming a relatively high resistance compared with rats and mice. Given that the hamster does respond to ferric oxide ($Fe_2O_3$)–benzo[*a*]pyrene (B*a*P) by developing significant incidences of respiratory cancer (Saffiotti et al. 1968; Blair 1974; Smith et al. 1975), but not to a number of other carcinogenic protocols including tobacco smoke (International Agency for Research on Cancer 1992), we can consider the hamster studies with diesel exhaust as negative. Pott and colleagues (1973), Montesano and coworkers (1970), and Feron and associates (1973) have also confirmed the sensitivity of the hamster lung to solid particles, sometimes in concert with organic compounds such as nitrosamines. Cynomolgus monkeys exposed to coal dust or diesel exhaust (2.0 $mg/m^3$) or a mixture (1 $mg/m^3$ of each) did not develop tumors, but concentrations of the substances and duration of exposure (less than 24 months) were insufficient to produce clear effects (Lewis et al. 1986, 1989a). These studies must be considered inadequate, rather than negative.

### Tumors at Sites Other Than the Lung

Only one of the several rat studies reported exposure-dependent increases in tumors at sites other than the lung. Iwai and associates (1986) noted a significant, three-fold increase in the incidence of splenic lymphoma and leukemia in female F344 rats exposed to diesel exhaust. Benign mammary gland tumors were also increased about three times in animals exposed to diesel exhaust. Two malignant sarcomas were detected in the exposed rats, but this study was compromised by the relatively small numbers of rats (24) in the treated groups. Ishinishi and coworkers (1988) also reported high incidences of lethal lymphoma and leukemia (25% to 40%) as well as mammary gland tumors in their test animals. Tumors of these types, however, appear to have been common in their untreated rats; more-

over, occurrence did not appear to be dependent on either exposure to diesel emissions or the concentration of the diesel exhaust particles. Mauderly and associates (1987) recorded two malignant tumors of the nasal cavity: one in a female control rat and the other in a male rat in the group exposed to the high-level diesel exhaust. The weight of evidence does not appear to support a role for inhaled diesel exhaust as an animal carcinogen in organs other than the lung in rodents.

### SUMMARY OF ANIMAL DATA

Animal data support the conclusion that whole diesel engine exhaust is a lung carcinogen in the rat, but not in the hamster; the evidence for the mouse is equivocal. In the rat, chronic inhalation of diesel exhaust or carbon black induced a dose-dependent increase in benign and malignant epithelial tumors when the concentrations of diesel exhaust or carbon black were sufficiently high (2.5 $mg/m^3$ or above). As noted earlier in this paper, such concentrations overwhelm the normal clearance mechanisms of the lung, resulting in accumulation of particles, macrophages, and their degradation products, as well as other cellular debris.

Four of the seven rat inhalation studies were performed with both males and females of the F344 strain with somewhat ambiguous results regarding gender sensitivity. Lung tumor incidences in the earlier study by Mauderly and associates (1987) were nearly identical in both genders in terms of dose of diesel exhaust particles and types of tumors induced. In the later study, however, Mauderly and associates (1994; Nikula et al. 1995) documented a greater sensitivity in the female rat, although this result may have been due in part to the consistently shorter survival time among males than among females. Although more adenocarcinomas were found in males than females at the highest level of exposure to heavy-duty diesel exhaust in the study by Ishinishi and coworkers (1988), the numbers are too small to draw definitive conclusions. A possibly better indicator in the Ishinishi study of relative sensitivity is the two-fold increase in adenomatous hyperplasia observed in females at each of the two highest concentrations of heavy-duty diesel exhaust. When rats killed after the end of the 24-month exposure to the high diesel-exhaust concentration were considered, a 96% incidence of total tumors in females was reported, compared with an incidence of 44% in males (Brightwell et al. 1989). The incidence of tumors in females was also at least two-fold higher than in males at earlier periods.

It can be concluded, therefore, that some evidence suggests that the female rat is more sensitive than the male (see below) to diesel exhaust, but no convincing evidence indicates that diesel exhaust or carbon black causes tumors in organs and tissues other than the lung.

## FACTORS AFFECTING CARCINOGENIC RESPONSE TO WHOLE DIESEL EXHAUST AND RELATED SUBSTANCES

### SPECIES DIFFERENCES IN SUSCEPTIBILITY

A potential for diesel exhaust to induce neoplasms was first demonstrated by Kotin and associates (1955) when these investigators used the solvent-extracted organic fraction of diesel exhaust to produce skin tumors in mice. About two decades passed before concern was expressed regarding the potential for diesel exhaust to cause human cancer. These concerns arose because of the predicted increase in the use of diesel engines in locomotives and other vehicles; this, coupled with an EPA report (Huisingh et al. 1978) that diesel exhaust particle extract was mutagenic in the Ames *Salmonella* assay, rekindled an interest in diesel exhaust and its potential effects. Shortly thereafter, in the early 1980s, chronic inhalation bioassays of rats, mice, and Syrian hamsters exposed to diesel exhaust, conducted in the United States, Germany, Japan, and Switzerland, produced generally consistent results (Mauderly 1992). The lung tumor incidence was increased in a dose-related manner in rats exposed repeatedly for 24 months or longer to diesel exhaust at weekly particle exposure rates above approximately 120 $mg/m^3 \cdot hr$. Some of these studies included groups exposed to filtered diesel exhaust, which demonstrated that the response required the presence of particles. The lung tumor response of mice to whole diesel exhaust was equivocal, and the responses of hamsters were uniformly negative.

It is not clear why hamsters are more resistant to diesel exhaust toxicity and carcinogenicity than rats and, to some extent, mice. In the studies of Heinrich and colleagues (1986), the data presented did not suggest any explanation for this phenomenon; body weights and mortality were comparable among the three species. Increases in the wet and dry weight of lungs were associated with diesel exhaust exposure in rats and mice after two years, and to a lesser extent in hamsters.

Hamsters exposed to diesel exhaust exhibited a small, insignificant decline in lung clearance after one year (half-time clearance of 75 versus 55 days, exposed animals versus control group). Hamsters developed lung tumors when exposed to inhalant plus particulate matter ($Fe_2O_3$) but not to inhalant alone, or if so, the incidence was insignificant (Saffiotti et al. 1968). In rats exposed to diesel exhaust, a highly

significant decrease in lung clearance was observed after only three months (half-time 2.5 times longer in exposed animals than in controls), after which there appeared to be adaptation with no further loss of capacity for half-time clearance.

Some biochemical analyses of lung lavage fluids showed that selected enzymes, collagen, and total protein contents were significantly increased in both hamsters and rats after one and two years of exposure to whole diesel exhaust. Total white blood cell counts in lung lavage were increased in both hamsters and rats; moreover, macrophage numbers were slightly increased in both species after one year.

Pretreatment of rats, mice, or hamsters with diethylnitrosamine, dibenz[*a*,*h*]anthracene (DB*ah*A), or B*a*P did not appear to inhibit or enhance development of chemically induced tumors in hamsters or mice (Heinrich et al. 1986). In rats, however, treatment with dipentylnitrosamine for the first 25 weeks of diesel exhaust exposure significantly increased both benign and malignant lung tumors. This study, although carefully performed and interpreted, does not clarify the question of special sensitivity of the rat lung relative to hamsters and mice. It does indicate, however, that the special sensitivity of the rat lung is in some way associated with the compromised alveolar clearance of particles, heavier deposits of inhaled particulate matter, and the sequelae to such events.

In another study (Brightwell et al. 1989), rats and hamsters were compared in their responses to chronic inhalation of gasoline and diesel engine exhausts. Results in this study were similar to those reported by Heinrich and associates (1986).

An interesting aspect of the hamster studies in both of the above referenced reports was the occurrence of diarrhea after about six months of exposure to diesel exhaust. This disease ("wet tail") has been encountered at some point by most investigators using this species. It usually subsides with time, treated or untreated, and generally is associated with animal husbandry practices (animal handling, housing in screen-bottom instead of solid-bottom cages, and sometimes type of feed) (Smith et al. 1975). In any case, the gastrointestinal disturbance did not appear to compromise the results of the two hamster studies.

## DUST OVERLOAD

The concept of dust overloading in the lungs of rats has been generally accepted by inhalation toxicologists for several years, and a number of investigators have reported on it (Bolton et al. 1983; Vostal 1986; Wolff et al. 1987; Lewis et al. 1989b; Morrow 1992, 1994; Oberdörster 1994; see also background paper by Green and Watson, this report). Most current inhalation studies of diesel exhaust factor dust overload into the design and interpretation of results. The overload phenomenon was seen initially as an experimental condition in which linear clearance kinetics became nonlinear (Vincent et al. 1985); however, later in chronic toxicity testing in rats, overloading became identified not only with protracted retention related to lung burden, but with other changes that seriously confounded toxicological interpretations. Dusts that were widely regarded as benign were shown to be capable of producing pathologic effects (e.g., fibrosis and tumors). Such effects were identical to those induced by highly toxic dusts when excessive amounts were persistently retained in the lungs (Lee et al. 1985). The most prominent effect of dust overload was a significant prolongation of alveolar macrophage–mediated particle clearance when the rats were exposed to highly insoluble, low-toxicity particles at exposure concentrations ranging from less than 10 to 100 mg/m$^3$ (Oberdörster 1994).

Chronic inhalation studies using a diversity of materials have revealed that a progressive prolongation of pulmonary dust retention apparently developed when the lung burden exceeded approximately 1 mg of dust per gram of lung tissue from rats that had undergone continued exposure (Morrow and Mermelstein 1988). At higher lung burdens of dust, approximately 10 mg/g of lung tissue, pulmonary dust clearance appeared to cease almost completely. Features of this progressive prolongation of pulmonary retention included aggregated alveolar macrophages filled with phagocytized dust particles, chronic inflammation, increased uptake of particles in the interstitial spaces, and alveolar cell hyperplasia. Alveolitis, granulomas, fibrosis, and pulmonary tumors increased with exposure time and with the severity of the overload condition (Lee et al. 1985; McClellan 1986; Vostal 1986; Morrow and Mermelstein 1988).

A hypothesis was developed (Morrow 1988) that the excessive levels of dust in the lungs led to excessive engulfment of particles by alveolar macrophages; after some undefined level of loading, the macrophages became progressively immobilized and aggregated. The onset of this loss of alveolar macrophages' ability to translocate occurred at a relatively constant lung burden for a variety of materials, suggesting that the overload condition appeared to have a generic quality. The description of average particle load per cell (described in Morrow 1988) is a convenient index of the overload condition in the rat.

One current explanation for suppression of particle transport by alveolar macrophages is the persistent elaboration of chemotactic and chemokinetic factors by alveolar macrophages after they die and disintegrate. Examples of factors elaborated by the alveolar macrophages include (1) growth factors for fibroblasts (Bitterman et al. 1982); (2) hydrogen

peroxide (Fisher and Bostick-Bruton 1982) and hydroxyl radicals (Ward et al. 1983); (3) neutrophil chemotactic factors (Hunninghake et al. 1978); (4) enzymes (e.g., lipoprotein lipase) (Okabe et al. 1984); (5) mediators for lymphoproliferative responses (Laughter et al. 1977); (6) immunomodulating factors (e.g., interleukin-1, interferon [Acton and Myrvik 1966]) and prostaglandins (Hsueh and Kuhn 1979); and (7) various mediators of host defense (e.g., complement components [Brain 1980] and leukotrienes [Hsueh and Sun 1982]).

Another hypothesis is that the continued particle deposition rate exceeds the macrophage clearance rate; macrophages that are actively ingesting particles become so full that they are immobile and are therefore unable to translocate.

## VARIABILITY IN THE PHYSICAL PROPERTIES OF PARTICLES

The reports by Kawabata and coworkers (1986) and Vostal (1986) fired the interest of other investigators to determine the carcinogenic effects of solid particles from diesel exhaust, free from organic compounds, and compare these results with exposure to other types of particles. Large studies were conducted by Heinrich and associates (1994b, 1995) and by Mauderly and colleagues (1994; Nikula et al. 1995), the results of which confirmed that carbon particles, virtually free of organic residues, were indeed carcinogenic for the rat lung (Tables 2, 3, and 4). The discussion below addresses various characteristics of particles that may bear on their carcinogenic properties. A key question relates to physical differences between carbon black particles and the carbonaceous core particles contained in diesel exhaust.

Table 7, some of which is from the Kawabata and associates study (1986), lists results comparing diesel exhaust particles and activated carbon with respect to tumor-inducing properties. Although diesel exhaust particles were more potent than carbon particles in the percentage of animals with tumors (75% versus 48%, respectively), this study established the tumor-inducing capabilities of carbon particles.

Additional data taken from Heinrich and coworkers' reports (1994a,b; 1995) confirm the observations of Kawabata and associates (1986) and the concepts put forth by Vostal (1986). As observed by Kawabata, the Heinrich data confirmed that carbon black (Printex 90) induced lung tumors after 10 months at a level close to that of diesel exhaust particles (17% versus 22%) (Tables 4 and 7). The 22% tumor incidence for diesel exhaust particles was observed after 24 months, however, at which time the tumor incidence for carbon black was only 8%. Whereas differences in physical characteristics between the diesel exhaust particles and carbon black were observed, these differences were not particularly notable.

Data in Table 7, from Lee and associates (1986), show that a $TiO_2$ particle lung load of 665 mg resulted in a tumor incidence of about 25%. In the Heinrich and colleagues (1992) study, a tumor rate of 32% was reported at a particle lung load of about 40 mg, after two years of exposure to an average particle concentration of 10 mg/m$^3$. The two types of $TiO_2$ apparently differ in carcinogenic potency, as similar tumor rates were observed with markedly different particle concentrations and particle burdens in the lungs.

In comparing diesel exhaust particles, one type of $TiO_2$, and carbon black (reviewed by Heinrich 1994), it seems appropriate to note characteristics that the dusts in these studies have in common: all the dusts are nearly insoluble in the lung; they have an aerodynamic diameter of 1.0 μm or less; they consist of agglomerates of primary particles in a size range of 10 to 50 μm; and their specific surface areas range from 40 m$^2$/g for $TiO_2$ P25 to 230 m$^2$/g for Printex 90 carbon black. All of the dusts caused deterioration of the alveolar lung clearance mechanisms that was dependent on exposure concentration, resulting in a pronounced accumulation of particles in the lung. It should be pointed out, however, that lung clearance was compromised after only three months of exposure to diesel exhaust particle concentrations of 0.8 mg/m$^3$, at which time the particle lung load was well below 1 mg.

Figure 1 illustrates that the dusts referred to above demonstrated a cumulative concentration-dependent increase in lung tumor incidence independent of type of particle, provided the experimental time was at least 24 to 30 months. The lowest exposure concentration of these ultrafine particles that led to a significant increase in lung tumor incidence was for the carbon core of diesel exhaust particles: 1.5 mg/m$^3$, inhaled for 18 hours per day, five days per week, for two years (reviewed by Heinrich 1994). The lowest lung load that caused a significant increase in lung tumor rate was 15 mg of Printex 90 carbon black particles or 10 mg/g of lung tissue.

As noted earlier, differences between the two types of $TiO_2$ in carcinogenic potency (Table 7) were also apparent with respect to toxicity. In this study (Heinrich 1994), the concentration of $TiO_2$ P25 at 10 mg/m$^3$ was near the maximum tolerated dose (increased mortality), higher than the $TiO_2$ used by Lee and associates (1985, 1986). Differences in the behavior of the $TiO_2$ used by Lee and associates and by Heinrich and colleagues (1992) can most likely be explained on the basis of different particle sizes between the two types, although this requires further investigation.

The data provided by Muhle and coworkers (1991) using a large-particle test toner that produced no increase in tumor incidence (Table 8) were in contrast to the observations of Heinrich and colleagues (reviewed by Heinrich

**Table 7.** Comparison of Tumor-Inducing Properties of Diesel Particles, Carbon Black Particles, and Titanium Dioxide in Rats[a]

| Particle | Exposure Conditions | Results: Lung Load (mg) | Results: Lung Tumor Incidence | Primary References |
|---|---|---|---|---|
| Diesel particles | Intratracheal instillation: total dose 10 mg | — | 31/42 | Kawabata et al. 1986 |
| Activated carbon | Intratracheal instillation: total dose 10 mg | — | 11/23 | |
| Diesel particles<br>MMAD[b]: 0.3 μm<br>Primary particle diameter: 10–50 nm<br>Extractable organic material: 40%<br>Benzo[*a*]pyrene: 4 ng/mg<br>1-Nitropyrene: 19 ng/mg | Inhalation: 7.5 mg/m$^3$, 18 hr/d, 5 d/wk for 24 mo | — | 22% | Heinrich 1989; Heinrich et al. 1994a; Heinrich 1994 |
| Carbon black (Printex 90)<br>MMAD: 0.6 μm<br>Primary particle diameter: 15 nm<br>Extractable organic material: 0.04%<br>Benzo[*a*]pyrene: 0.6 pg/mg<br>1-Nitropyrene: < 0.5 pg/mg | Inhalation: 6 mg/m$^3$, 18 hr/d, 5 d/wk for 10 or 20 mo | — | 17% at 10 mo; 8% at 20 mo | |
| Diesel particles[c]<br>Primary particle diameter: 10–50 nm | Inhalation: 7.5 mg/m$^3$, 18 hr/d, 5 d/wk for 24 mo<br>Cumulative exposure: 61.7 g/m$^3$ • hr | 63.9 | 22% | Heinrich et al. 1992; Heinrich et al. 1994b |
| Titanium dioxide (P25)[c]<br>Primary particle diameter: 15–30 nm | Inhalation: 18 hr/d, 5 d/wk for 24 mo[d]<br>Cumulative exposure: 88.1 g/m$^3$ • hr | 39.3 | 32% | |
| Carbon black (Printex 90)[c]<br>Primary particle diameter: 15 nm | Inhalation: 18 hr/d, 5 d/wk for 24 mo[e]<br>Cumulative exposure: 102.2 g/m$^3$ • hr | 43.9 | 39% | |
| Titanium dioxide[c]<br>compact particles (1.2 μm diameter) | Inhalation: 5 mg/m$^3$<br>10 mg/m$^3$<br>50 mg/m$^3$<br>250 mg/m$^3$ | 3.2<br>27.0<br>124.0<br>665.0 | 2%<br>—<br>—<br>25.8% | Bellmann et al. 1991; Muhle et al. 1991; Lee et al. 1985, 1986 |
| Titanium dioxide<br>Aggregates of ultrafine primary particles (15–30 nm diameter) | Inhalation: 10 mg/m$^3$ (average) | 39.0 | 32% | Heinrich et al. 1992 |

[a] Data are from Heinrich (1994).

[b] Mass median aerodynamic diameter.

[c] MMAD for all three particle types: 0.3 to 0.8 μm.

[d] Exposed to 7.5 mg/m$^3$ for 4 months, 15 mg/m$^3$ for 4 months, and 10 mg/m$^3$ for 16 months.

[e] Exposed to 7.5 mg/m$^3$ for 4 months and 12 mg/m$^3$ for 20 months.

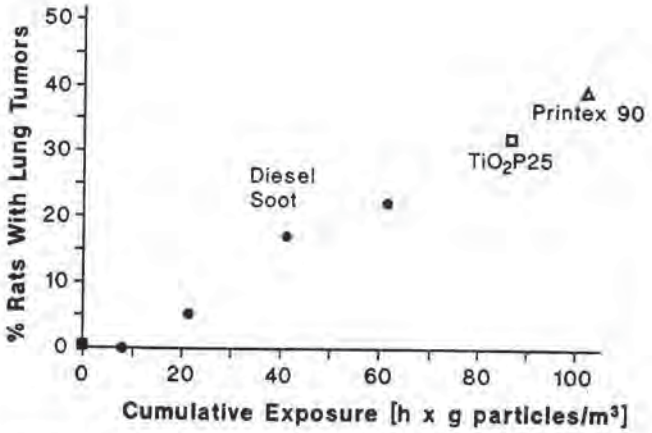


**Figure 1. Lung tumor incidence and cumulative exposure to carbon black (Printex 90), titanium dioxide ($TiO_2$) P25, and diesel exhaust particles (Heinrich et al. 1986a, 1992).** (Reprinted with permission from International Life Sciences Institute Press, as published in Heinrich 1994).

1994) that a lung load of 15 mg of Printex 90 carbon black produced a significantly increased lung tumor incidence of 17%.

Another study (Martin et al. 1977) used rats and a high concentration of a dust of relatively large particle size (coal dust) (Table 8) that led to a particle lung load of almost 100 mg and to an increased lung tumor incidence of 11%; that is, 4 tumor-bearing animals out of 36. This was the first report of the induction of lung tumors after inhalation exposure to a high concentration of coal dust.

Studies using such relatively large dust particles (test toners, coal dust, and volcanic dust) were terminated after 24 or 26 months (Table 8). Only a limited percentage of the dust samples were in the respirable size range, and lung tumors were induced only when particle concentrations reached 200 mg/m$^3$ or higher and when particle lung load after two years of exposure reached at least 100 mg (Wehner et al. 1986).

## ROLE OF EMISSION COMPONENTS IN CARCINOGENIC ACTIVITY

Unfiltered diesel exhaust contains two main components: (1) a vapor-phase mixture of relatively low molecular weight organic chemicals, and (2) a particulate phase of carbonaceous particles to which higher molecular weight organic compounds are adsorbed; the latter includes the well-known carcinogenic polycyclic aromatic hydrocarbons (PAHs) such as B$\alpha$P. Because of their mutagenic and carcinogenic potency, initial research in this area focused on the role of PAHs in lung tumors induced by diesel exhaust. More recently, the question has been raised whether almost insoluble solid dust particles, generally regarded as toxicologically inert, may exert a carcinogenic or cocarcinogenic effect in the respiratory tract.

### EFFECTS OF FILTERED AND UNFILTERED DIESEL EXHAUST

Prior to studies with particles relatively free of organic contaminants, a number of studies were conducted comparing filtered and unfiltered diesel exhaust. Table 9 summarizes several chronic inhalation studies in which one group of animals was exposed to diesel exhaust and another group to filtered exhaust from which the particulate phase was removed. In the four studies with rats, no lung tumors were induced in any of the animals exposed to filtered exhaust, whereas significant incidences were reported in all treated groups exposed to unfiltered exhaust. This clearly demonstrates the primary importance of the particulate phase of

**Table 8.** Comparison of Incidence of Lung Tumors and Lung Load in Rats After Chronic Inhalation Exposure to Compact Particles of Larger Size[a]

| Particle Tested | Exposure (mg/m$^3$) | Lung Load (mg) | Tumor Incidence[b] (%) | Primary Reference |
|---|---|---|---|---|
| Test toner | 5 | 16 | 2 | Muhle et al. 1991 |
| Coal dust | 200 | 96 | 11 | Martin et al. 1977 |
| Coal dust | 5 | 1 | 4 | Lewis et al. 1989b |
| Volcanic ash | 5 | 32 | 0 | Wehner et al. 1986 |
| Volcanic ash | 50 | 207 | 4 | Wehner et al. 1986 |

[a] Diameter greater than 1 µm. Data are from Heinrich (1994).

[b] Spontaneous lung tumor incidence was 1% to 3%.

diesel exhaust in lung tumor causation in rats, but it does not indicate whether the organic compounds bound to the carbonaceous particles, the particles themselves, or a combination of the particles along with the bound compounds are responsible for the tumorigenic response.

In apparent contrast to the results in rats, an early chronic inhalation study with female NMRI mice showed that both filtered diesel exhaust (with the particles removed) and unfiltered diesel exhaust induced a significant elevation in lung tumor incidence (Heinrich et al. 1986). A more recent study from the same laboratory using the same mouse strain and a higher concentration of diesel exhaust indicated no elevation of tumor incidence with unfiltered diesel exhaust (Heinrich et al. 1995). However, compared with clean air exposure, the filtered diesel exhaust induced an increase in lung tumor incidence of more than 50%, but this response was not significant ($p = 0.053$). The NMRI mouse strain is characterized by a high spontaneous lung tumor incidence, and the positive results in the first study were attributed to an unusually low tumor incidence in the control animals (International Agency for Research on Cancer 1989a). A companion study using a mouse strain with a very low incidence of spontaneous lung tumors (C57BL/6N) indicated no elevation in lung tumors with filtered or unfiltered diesel exhaust (Heinrich et al. 1995).

Clearly, mice are much less sensitive to diesel exhaust than rats. In common with rats, however, mice exposed to unfiltered diesel exhaust exhibited a four-fold increase in adenomatous hyperplasia (64% incidence) relative to those exposed to filtered exhaust or clean air (15% or 5% inci-

**Table 9.** Effect of Particle Removal on Carcinogenicity of Diesel Exhaust in Rats and Mice

| | Exposure Conditions | | | Lung Tumors (%) | | | |
|---|---|---|---|---|---|---|---|
| Strain (Gender) | Engine Size (L) | Exposure | Particle Concentration ($mg/m^3$) | Benign | Malignant | Total | Reference |
| F344 rats (F) | 2.4 | Control | 0 | 4.5 | — | 4.5 | Iwai et al. 1986 |
| | | Unfiltered | 4.9 | 15.8 | 26.3 | 42.1[a] | |
| | | Filtered | 0 | 0 | 0 | 0 | |
| Wistar rats (F) | 1.6 | Control | 0 | — | — | 0 | Heinrich et al. 1986 |
| | | Unfiltered | 4.2 | (14.7-16.8)[b] | 1.1 | 15.8[a] | |
| | | Filtered | 0 | — | — | 0 | |
| F344 rats (M) | 11 | Control | 0 | — | — | 0 | Ishinishi et al. 1988 |
| | | Unfiltered | 3.0 | — | 12.5 | 12.5[a] | |
| | | Filtered | 0 | — | — | 0 | |
| F344 rats (M+F) | 1.5 | Control | 0 | — | — | 1.2[c] | Brightwell et al. 1989 |
| | | Unfiltered | 6.6 | — | — | 38.5[a,c] | |
| | | Filtered | 0 | — | — | 0 | |
| NMRI mice (F) | 1.6 | Control | 0 | 10.7 | 2.4 | 13.1 | Heinrich et al. 1986 |
| | | Unfiltered | 4.2 | 14.5 | 17.1 | 31.6[a] | |
| | | Filtered | 0 | 11.8 | 19.4 | 31.2[a] | |
| NMRI mice (F) | 1.6 | Control | 0 | 25 | 8.8 | 30 | Heinrich et al. 1995 |
| | | Unfiltered | 4.5 | 18.3 | 5 | 23 | |
| | | Filtered | 0 | 31.7 | 15 | 46.7[d] | |
| C57BL/6N mice (F) | 1.6 | Control | 0 | — | — | 5.1[c] | Heinrich et al. 1995 |
| | | Unfiltered | 4.5 | — | — | 8.5[c] | |
| | | Filtered | 0 | — | — | 3.5[c] | |

[a] Significantly different from control ($p < 0.05$).

[b] Range of tumor incidence based on possibility of multiple tumors present in one animal.

[c] Insufficient information to discern proportion of benign and malignant tumors.

[d] Not significantly different from control ($p > 0.05$).

dence, respectively) (data not shown in Table 9). Also, fibrotic loci (an indication of damage to lung tissue by particles) were present almost exclusively in lungs of animals exposed to unfiltered exhaust. Fibrosis and hyperplasia were associated with the eventual development of lung tumors in rats (Ishinishi et al. 1986, 1988), but what this finding represents is unclear.

## EFFECTS OF SOLID PARTICLES

The first experimental evidence that inert particles such as carbon may cause tumors in the rat lung was reported by Kawabata and associates (1986), who observed that 49% of 23 rats treated intratracheally with a total dose of 10 mg of activated carbon developed lung tumors. In another group of rats, 77% developed lung tumors after treatment with 10 mg of diesel exhaust particles. One tumor-bearing animal was observed in 23 solvent-control rats. The extract of the activated charcoal was stated to be negative in the Ames test, but no data were given on the amount and composition of the extractable organic material (Table 8). Following Kawabata's report (1986), Heinrich and coworkers (1994b, 1995) conducted a long-term inhalation study with diesel exhaust, carbon black particles, irritant gases, or a mixture of these; some of the results are shown in Table 3. Diesel exhaust particles as well as Printex 90 carbon black used in this study consisted of agglomerates of primary particles in the size range below 50 nm. Rats were exposed to concentrations of commercially available carbon black of 6 $mg/m^3$ for 18 hours per day, five days per week, for either 10 or 20 months, followed by a clean air time of 20 or 10 months, respectively. Rats in both 10- and 20-month exposure groups had a significant increase in lung tumor incidence. In one case, however, an unexplained incidence observed after 10 months was higher than at 20 months. At the end of the 10-month exposure period, the lungs contained 10 mg of carbon black and the tumor rate in this group (group 3) was 20%, thus demonstrating that carbon black, devoid of detectable organic material, can induce lung tumors in rats after inhalation exposure.

In an effort to determine whether a noncarbon dust particle, such as $TiO_2$, may have carcinogenic effects in the lung of the rat, Heinrich and coworkers exposed rats for two years to $TiO_2$ P25 (Heinrich et al. 1992; Heinrich 1994). The size of the primary particles of this $TiO_2$ dust was about 15 to 30 nm. Other groups of rats were exposed to Printex 90 carbon black or to diesel engine exhaust. Exposure was for 18 hours per day, five days per week, for 24 months; all three groups had a significantly increased lung tumor rate (Table 4).

Rats exposed to dust particles as described above exhibited a cumulative concentration-dependent increase in the incidence of lung tumors after 30 months (Figure 1). The lowest exposure concentration of these ultrafine particles that led to a significantly increased lung tumor incidence was a concentration of the carbon core of diesel exhaust particles of 1.5 $mg/m^3$. The lowest lung particle load in rats with a significantly increased lung tumor rate was 15 mg of Printex 90 carbon black particles or 10 mg/g of lung tissue (Heinrich 1994).

Muhle and coworkers (1991) exposed rats to a test toner consisting of 90% copolymer and 10% highly purified carbon black with an aerodynamic diameter of 4 μm. Exposure to a concentration of 16 $mg/m^3$ resulted in a particle lung load of 16 mg after 24 months of exposure, but no increased lung tumor incidence was observed after an experimental time of 26 months (Table 8).

One other inhalation study with rats (Martin et al. 1977) reported a significantly increased lung tumor rate after exposure to 200 $mg/m^3$ of coal dust containing less than 0.4% quartz for 24 months. This led to a particle lung load of almost 100 mg and to an increased lung tumor rate (Table 8), which was the first demonstration of the induction of lung tumors after exposure to a high concentration of coal dust particles. Later studies, however, failed to confirm the tumor-inducing properties of coal dust (Wehner et al. 1986; Lewis et al. 1989a). It is still uncertain whether coal dust particles induce lung cancer in rodents, but adequate studies may not have been done.

The results of Mauderly and associates (1994; Nikula et al. 1995) and Heinrich and colleagues (1994b, 1995) extend experiments in the rat to comparisons of the carcinogenicity of whole diesel exhaust and carbon black particles. These new additions to the database on pulmonary carcinogenicity in rats may have further relevance for predicting lung cancer risk in humans.

After 3, 6, 12, 18, and 23 months of exposure (Mauderly et al. 1994; Nikula et al. 1995), rats were killed, and lung and lung-associated lymph node burdens of particles, lung weight, bronchoalveolar lavage indicators of inflammation, chromosome injury in circulating lymphocytes, and histopathology were analyzed. The relationship of particle dose to particle clearance was determined using clearance of radiolabeled carbon black particles and sequestration of fluorescent microspheres inhaled acutely after 3 and 18 months of chronic exposure. Tumors were transplanted into athymic mice to examine the growth characteristics of lung neoplasms.

In all parameters measured, exposures to diesel exhaust and carbon black caused nearly identical effects. Dose-related slowing of particle clearance was observed for both diesel exhaust and carbon black by three months of exposure; progressive accumulation of particles in lungs and lymph nodes was also evident at this time. Lavage demon-

strated persistent inflammation and cytotoxicity in the lung; histologic evaluation of the lungs demonstrated nonneoplastic changes consisting of inflammation, epithelial proliferation, and fibrosis, all of which were progressive and dose related (Table 6). Chromosome damage in circulating lymphocytes was not associated with exposure, but the incidence of primary lung neoplasms was increased significantly in all exposed groups in a dose-related manner (Table 2). The types of neoplasms resulting from diesel exhaust and carbon black exposure were identical, with no significant difference between the carcinogenic potencies of the two materials observed. Neoplasms of the same type from both exposures had similar growth characteristics when transplanted into athymic mice. Sequestration and translocation of tracer particles were similar in rats exposed to diesel exhaust and rats exposed to carbon black.

The nearly identical carcinogenic and noncarcinogenic effects associated with diesel exhaust and carbon black exposure suggested that organic compounds associated with diesel exhaust particles may not play an important role in the pulmonary carcinogenicity of diesel exhaust in rats exposed chronically at high concentrations. This is in direct opposition to the concepts of most earlier investigations. Although additional organic residues might have been extracted from the carbon black by more thorough procedures, the findings suggest that the pulmonary carcinogenicity of diesel exhaust in heavily exposed rats probably does not occur by the chemical mechanisms that have been previously hypothesized. In the past, these presumed mechanisms were based on the mutagenicity of extracts of diesel exhaust particles. Although these findings do not prove that the mutagenic activity associated with diesel exhaust particles does not contribute to lung cancer risk in humans exposed at lower rates for longer times, they do suggest that it is inaccurate to extrapolate unit lung cancer risks for humans from rat carcinogenicity data using the amount of deposited organic material as the basis for comparison.

## EFFECTS OF ORGANIC COMPOUNDS

Despite convincing evidence now emerging from a number of laboratories (Heinrich et al. 1994b, 1995; Mauderly et al. 1994; Nikula et al. 1995) that the carbonaceous core particle of diesel exhaust may play the leading role in lung cancer in rodents, it is too early in the evaluation of available data to exclude organic chemicals as significant contributors to oncogenesis. Tumor incidences were similar in rats exposed to diesel exhaust and in rats exposed to carbon black particles free of organic compounds in two major studies; however, it is not clear from the data provided whether there were qualitative or quantitative differences in the types of tumors produced by the exposures, although it appears that they were comparable. In addition, the degree to which these tumors may have expressed malignant tendencies is unclear, or if like results would be observed in the mouse under comparable exposure conditions.

Diesel exhaust contains a large number of compounds belonging to many different chemical classes (International Agency for Research on Cancer 1989a,b). Major classes of chemicals identified in diesel exhaust include aliphatic hydrocarbons; benzene and phenol derivatives; PAHs and their alkyl-substituted derivatives; nitro-PAHs; oxygenated PAHs, including ketones, carboxaldehydes, acid anhydrides, quinones, and hydroxy derivatives; and nitrogen-containing heterocyclic compounds (Schuetzle 1983). Many of these PAHs, PAH derivatives, and heterocyclic compounds are well-known mutagens and animal carcinogens (International Agency for Research on Cancer 1973, 1983, 1989a,b).

It has been confirmed that the organic compound fractions present in diesel emissions are tumorigenic in laboratory animals under appropriate conditions of exposure. These experiments, however, were usually performed with animals exposed by routes other than inhalation and generally at high concentrations. Thus the results are of little use in estimating human risk from exposure to the organic chemical component of diesel exhaust.

### Lung Implantation Studies

One approach to the identification of chemical carcinogens in diesel exhaust is to separate chemically the classes of compounds extracted from the mixture and test the individual fractions for carcinogenicity. Grimmer and coworkers (1987a) fractionated exhaust condensate from a 3.0-L diesel engine and tested the component parts in a rat lung implantation assay; the test substance was dissolved in warm beeswax and injected into the left lobe of the lung in anesthetized three-month-old female rats, where it slowly diffused from the congealed beeswax implant over the two-year course of the assay (Deutsch-Wenzel et al. 1983). The results are summarized in Table 10. All of the carcinogenic activity was associated with the hydrophobic fraction, which, upon further separation, yielded four subfractions representing different classes of PAHs and their derivatives. The subfractions that contained nonaromatic compounds, polynuclear aromatic compounds (PACs) containing two to three rings, and the polar-PACs were devoid of carcinogenic activity, even though they contained more than 95% by weight of the material in the original carcinogenic fraction. The majority of the carcinogenic activity resided in the subfraction containing PAHs with four to seven rings.

In general, the major carcinogenic components in other combustion-derived emissions such as gasoline engine exhaust (Grimmer et al. 1983, 1984a), emissions from hard coal–fired residential furnaces (Grimmer et al. 1985, 1987b), and cigarette sidestream smoke (Grimmer et al. 1988) also were found consistently in this subfraction, regardless of whether a rat lung implantation assay or a mouse skin-painting assay was used to evaluate carcinogenicity. The subfraction containing PAHs with four to seven rings includes most of the well-known classic PAH carcinogens such as B*a*P, benz[*a*]anthracene, and the dibenzanthracene (DBA) and dibenzopyrene (DBP) series of isomers (International Agency for Research on Cancer 1983).

Particularly noteworthy, given the attention that nitro-PAHs have received because of their potent mutagenicity in bacteria and their high concentrations in diesel emissions (Rosenkranz and Mermelstein 1985; Tokiwa and Ohnishi 1986), is that the nitro-PAH subfraction induced less than 20% of the lung tumors (actual incidence 2.8%) attributed to the PAHs with four to seven rings (Table 10). This indicated that the nitro-PAHs made a low overall contribution to the carcinogenic activity of diesel particle extract under the conditions of the experiment.

### Skin-Painting Studies

Comparative dose-response data from skin-painting studies in mice using a tumor promotion protocol with tetradecanoyl phorbol acetate (TPA) were obtained from particle extracts from several diesel engines, as well as from a spark-ignition gasoline engine, roofing tar, and coke oven emissions (Nesnow et al. 1982a,b, 1983). Selected data in Table 11 summarize the incidence and number of skin tumors at a fixed dose of 2 mg of extract emissions, although testing was performed with extract doses ranging from 0.1 to 10 mg. Information on the B*a*P concentration and the amount of organic material extractable from the emission particles is included as well.

**Table 10.** Carcinoma Incidence Following Lung Implantation of Fractions from Light-Duty Diesel Engine Exhaust (Particle Extract Plus Vapor-Phase Condensate)[a]

| Fraction | % of Total Condensate by Weight | Dose (mg) | Carcinoma Incidence (%) |
|---|---|---|---|
| Control | — | — | 0 |
| 1) Hydrophilic | 25.0 | 6.7 | 0 |
| 2) Hydrophobic | 75.0 | 20.0 | 14.2 |
| a) Nonaromatics + 2- to 3-ring PACs | 72.0 | 19.22 | 0 |
| b) 4- to 7-ring PAHs | 0.8 | 0.21 | 17.1 |
| c) Polar-PACs | 1.1 | 0.29 | 0 |
| d) Nitro-PAHs | 0.7 | 0.19 | 2.8 |
| Subtotal | 74.6 | 19.91 | 19.9 |
| 3) Reconstituted hydrophobic (2a, 2b, 2c, 2d) | 74.5 | 19.91 | 20.0 |

[a] Data are from Grimmer et al. (1987a).

Overall, there was a rough correlation between emission potency as a skin tumorigen and its B*a*P concentration. The three most potent emission extracts contained the three highest concentrations of B*a*P (Table 11). The incidence of skin tumors, however, is not a linear function of B*a*P concentration (Nesnow et al. 1983). As an example, the coke oven topside sample was three to five times more potent than the Nissan diesel extract, yet it contained less than one-half the amount of B*a*P. These results could be due to differences in the concentrations of other carcinogens and perhaps to synergistic or inhibitory effects among different components of these extracts.

The extract from the Nissan engine exhaust was the most potent of the diesel samples in the skin-painting assay (Table 11), an observation that subsequently has been attributed to suboptimal function due to improper tuning of the engine (Nesnow et al. 1983).

Dose-response data were obtained only for particle extracts from the Nissan and Volkswagen engines (Nesnow et al. 1982a,b, 1983). Tumor incidence with the Oldsmobile engine sample declined at the highest dose tested, a result attributed to sample toxicity. The particle extract from the heavy-duty Caterpillar engine was essentially inactive. Also, the Nissan engine extract, in contrast to the coke oven mains and roofing tar samples, was inactive as a complete carcinogen when the skin-painting assay was run in the absence of TPA (Nesnow et al. 1983). Kunitake and coworkers (1988), in a tumor initiation protocol in the presence of TPA, showed only small, insignificant increases in skin tumors following dermal application of up to 45 mg of particle extract from a heavy-duty or a light-duty diesel engine. It appears that particle extracts from properly functioning diesel engines are not strong tumorigens in the mouse skin-painting assay, even when the assay is run in the more sensitive tumor initiation mode with TPA promotion.

### Estimation of Comparative Tumorigenic Potencies of Polycyclic Aromatic Hydrocarbons and Nitro–Polycyclic Aromatic Hydrocarbons

Among the carcinogenic PAHs, B*a*P is the accepted reference standard by which the potency of other PAHs is

often measured. Benzo[*a*]pyrene is a ubiquitous environmental contaminant present in air, soil, and water (Grimmer and Pott 1983; International Agency for Research on Cancer 1983). It is produced from the incomplete combustion of fossil fuels, wood, and tobacco products, and is often present in human foods after cooking or other forms of heat processing. It is considered to be a powerful carcinogen and, unlike the majority of other carcinogens, produces tumors in many organs and tissues in virtually every animal species in which it has been tested, regardless of the route of administration (International Agency for Research on Cancer 1973). Furthermore, some studies have measured the formation of DNA adducts from B*a*P as a biomarker to indicate damage to DNA and as a measure of exposure to PAH-containing emissions in human subjects (Harris et al. 1987; Perera 1987).

Thus it is noteworthy that B*a*P apparently was consistently responsible for only a small fraction of the carcinogenic activity associated with a number of combustion emissions. In general, the fraction of B*a*P activity was much lower in the rat lung implantation assay than in the mouse skin-painting assay for reasons that are not clear. As examples, B*a*P was credited with 1.4% to 2.8% of the carcinogenic activity in emissions from hard coal–fired residential furnaces or from a gasoline engine when the lung implantation assay was used to determine carcinogenic activity (Grimmer et al. 1984a, 1987b). It accounted for 6% to 12% of the activity when these emissions were evaluated in the mouse skin-painting assay (Grimmer et al. 1983, 1984b, 1985).

A major question now is, if B*a*P was responsible only for such a small fraction of carcinogenic activity, what other compound, or compounds, contributed significantly to the remaining activity? This question has obvious relevance not only for diesel exhaust, but also for PAH-containing emissions from other combustion and pyrolysis sources, and is discussed further in Appendix C.

## EVIDENCE FOR TUMOR PROMOTERS OR COCARCINOGENS IN DIESEL EXHAUST

There are many examples of weak carcinogens or noncarcinogenic substances that amplify tumor response in animals when administered before, with, or after treatment

**Table 11.** Skin Tumor Initiation Potencies of Diesel Engine Exhaust and Other Combustion Product Emissions[a]

| Sample | %Extractable Particles | Benzo[*a*]pyrene (ng/mg extract) | Skin Tumors[b] Incidence (%) Male | Incidence (%) Female | Number per Mouse Male | Number per Mouse Female | Dose for 50% Tumor Incidence (mg) |
|---|---|---|---|---|---|---|---|
| Control | — | — | 8 | 5 | 0.08 | 0.05 | — |
| Caterpillar diesel (1972, model 3304)[c] | 27 | 2 | 11 | 5 | 0.11 | 0.05 | NC[d] |
| Volkswagen Rabbit diesel[c] (turbocharged prototype) | 18 | 26 | 21 | 14 | 0.24 | 0.17 | NC |
| Mustang II gasoline (1977, 301 $in^3$) | 43 | 103 | 22 | 21 | 0.24 | 0.23 | NC |
| Oldsmobile diesel[c] (1978, 350 $in^3$) | 17 | 2 | 20[e] | 40[e] | 0.35[e] | 0.40[e] | NC |
| Roofing tar pot (external propane burner) | >99 | 889 | 36 | 37 | 0.62 | 0.45 | 1.8–2.1 |
| Datsun-Nissan diesel[c] (1973, 220C preproduction) | 8 | 1,173 | 66 | 58 | 1.10 | 1.60 | 1.5-1.6 |
| Coke oven (topside) | 7 | 478 | 95 | 90 | 4.00 | 3.50 | 0.30-0.42 |

[a] Data are from Nesnow et al. (1982b, 1983).

[b] Tumor data are from the 2-mg sample only.

[c] All diesel engines were fueled with the same batch of No. 2 diesel fuel.

[d] NC = not calculated.

[e] No dose-response relation in activity between 1.0 and 10.0 mg of sample.

with a known carcinogen; these are referred to as promoters or cocarcinogens. Promoters are noncarcinogenic substances, which when administered after pretreatment with a carcinogen or tumor initiator increase tumor yield by stimulating the proliferation of cells already transformed to a malignant or premalignant state (Williams and Weisburger 1991). Cocarcinogens are substances that may or may not be carcinogens themselves, but increase tumor frequency or incidence (or both) when administered with a carcinogen. Cocarcinogenesis is distinguished from tumor promotion because the order of treatment with the two chemicals does not affect the increase in tumor yield; promoters are inactive when administered before a carcinogen.

No information has been published on the possible promoting activity of diesel emissions in laboratory animal experiments. Zamora and colleagues (1983), however, compared the activity of a diesel particle extract with the potent activity of the tumor promoter TPA using a variety of in vitro measures of promotion in cultured animal cells. Results of these studies suggested that the diesel particle extract contained substances that acted as weak promoters by slightly increasing the frequency of cell transformation by another carcinogen; some specific cell surface properties were altered as well. Most of the changes observed were dose dependent, but the results must be interpreted with caution because the effects were seen at extract concentrations close to those that were cytotoxic.

Possible cocarcinogenic effects were sought by Heinrich and coworkers (1986) and Brightwell and associates (1989) in conjunction with their investigations on the carcinogenicity of inhaled diesel exhaust in hamsters, rats, and mice. Experimental animals before or during exposure to exhaust or clean air were treated with a compound known to induce respiratory tract tumors. The rationale for this approach was to treat animals with sufficient amounts of compound to induce a small but significant number of tumors, and then evaluate whether exposure to diesel exhaust increased the tumor yield.

Hamsters treated with either diethylnitrosamine (a trachea and lung carcinogen in this species when administered by subcutaneous injection) or B*a*P (a lung carcinogen following intratracheal instillation) did not exhibit any significant changes in the number of these tumors after exposure to diesel exhaust (Heinrich et al. 1986; Brightwell et al. 1989). In the Heinrich study, however, B*a*P and diethylnitrosamine treatment induced a tumor incidence too low (less than or equal to 10%) to assess adequately a cocarcinogenic effect if present. Diesel exhaust alone did not induce tumors in hamsters as shown by a number of other studies.

No consistent cocarcinogenic effects were observed in mice given intratracheal instillations of either B*a*P or dibenz[*a*,*h*]anthracene prior to exposure to diesel emissions (Heinrich et al. 1986). Both of these compounds are lung carcinogens in this species and induced high incidences of tumors that in some cases were actually decreased (anticarcinogenic effect) by subsequent diesel exhaust exposure. The investigators viewed these findings as inconsistent and erratic, however. Filtered and unfiltered diesel exhaust each induced lung tumors in the mouse (Heinrich et al. 1986), but incidences were relatively low compared with those in controls, and the study did not clarify the question of cocarcinogenic effects in this species.

Heinrich and coworkers (1986) injected rats subcutaneously with two dose levels of dipentylnitrosamine during the first 25 weeks of exposure to diesel exhaust. This compound induced high rates (approximately 90%) of tumors in the lung (primarily adenocarcinomas) and lower incidences (25% to 50%) of tumors (predominantly papillomas) in the upper respiratory tract (nasal cavity, larynx, and pharynx). Exposure to unfiltered diesel exhaust did not have any effect on overall lung tumor incidence, but did result in significant increases (2- to 10-fold) in squamous cell carcinoma. No information was provided as to whether the incidence of other types of lung tumors was affected. Treatment with two dose levels of dipentylnitrosamine significantly lowered the incidence of upper respiratory tract tumors by two-thirds. In addition, the incidence of liver and kidney tumors was low (up to 15%) and remained unaffected by diesel exhaust exposure.

With respect to cocarcinogenicity or promotion, limited evidence suggests that under certain conditions diesel exhaust elicited changes in the number and types of respiratory tract tumors induced by concurrent or prior treatment with known carcinogens. So far, consistent results have been obtained only for the rat, the only species in which corroborative evidence for the carcinogenicity of diesel exhaust has been observed. The results to date suggest that these effects are complex and produce both increases and decreases in tumor yields, depending on conditions of the experiment. Furthermore, the effects appear to be related to tumor type and site. Inconsistent and erratic results were obtained in the mouse, and the single adequate study in hamsters showed no effect; these results are consistent with results from other inhalation carcinogenesis studies.

Limited evidence in cultured cells indicated that extracts of diesel particles contain weak promoters, but these results were obtained with near-toxic doses and must be viewed with caution. No two-stage promotion studies have been performed in mouse skin-painting assays to confirm whether these extracts contain classic tumor promoters.

## DISCUSSION AND CONCLUSIONS

As noted in the Introduction section of this background paper, diesel engine emissions are complex mixtures that comprise thousands of organic and inorganic substances, the balance of which appear to be affected by a number of factors. These include the type of fuel, engine design, properties of the lubricating oil, and conditions under which the engine is operated, along with additional environmental elements. This paper presents the available evidence for animal carcinogenicity of diesel emissions, discusses the roles played by different emissions components in carcinogenic activities, and examines data to clarify whether diesel emissions contain promoters or cocarcinogens that may contribute to their tumor-inducing properties. Additional aspects of this paper include a description of the nature of tumors, including cancer; types of tumors associated with diesel exhaust, diesel exhaust particles, and other solid particles and organic compounds contained in diesel emissions; differing means and routes of exposure; estimations for comparative tumorigenic potencies of organic compounds contained in diesel exhaust or adsorbed to the carbonaceous particulate core; carcinogenicity of solid particles in experimental animals; evidence for promoters or cocarcinogens; and some considerations of animal species differences in sensitivity to carcinogenic effects of diesel engine exhaust and selected components.

A comprehensive review of available data on chronic exposure of laboratory animals to diesel exhaust (Mauderly 1992) and data compiled since (Heinrich et al. 1994b, 1995; Mauderly et al. 1994; Nikula et al. 1995) confirm that whole diesel exhaust is a lung carcinogen for the rat; the data for mice are equivocal, and the Syrian hamster has not developed lung tumors in any of the studies reported thus far.

There is no longer any doubt that whole diesel exhaust will induce lung cancer in rats if the exposure is of sufficient concentration and duration. Diesel exhaust particle concentrations of approximately 3 $mg/m^3$ or higher and exposure for 24 months or more appear to be adequate. The carcinogenicity has been confirmed in two strains of rats (Wistar and F344) in at least five different laboratories. At least seven different studies with rats have reported that tumors of epithelial origin were induced at significant incidences, ranging generally from about 5% to from 30% to 40%, though more often the latter in recent studies. The tumor types included both benign and malignant forms, including adenomas (benign) and adenocarcinomas, squamous cell carcinomas, and adenosquamous carcinomas (malignant). The malignant forms tended to exceed benign types overall, and squamous cell carcinomas were in higher incidence in one laboratory (Heinrich et al. 1994b, 1995), whereas adenocarcinomas were more frequent in another laboratory (Mauderly et al. 1994; Nikula et al. 1995).

Overall, the female rat appeared to be more sensitive than the male to the tumorigenic effects of whole diesel exhaust; in some cases the incidence of tumors, most of which were in the adenocarcinoma category, was three times higher in females (Mauderly et al. 1994; Nikula et al. 1995); however, this may have been a result of decreased survival of males. Because the large studies by Heinrich and colleagues (1994b, 1995) used only female rats, a gender comparison could not be made.

Prior to the well-designed study by Mauderly and coworkers (1994; Nikula et al. 1995), a definitive answer to the question of gender differences was not available. Three of the five previous inhalation studies used both males and females of the F344 strain, but the results were inadequate to answer the question of gender differences. The Mauderly study reported in 1987 found nearly identical tumor incidences in males and females, in terms of dose of diesel exhaust particles and types of tumors induced; however, as noted in the later, larger study (Mauderly et al. 1994), females had higher incidences than male rats, but, as noted above, survival may have been a factor. While suggestive of a gender difference, this question requires further attention.

The recent studies of Heinrich and associates (1994b, 1995) and Mauderly and coworkers (1994) confirmed that carbon black of different types with different physical properties is also carcinogenic for at least two strains and both genders of rats. This important observation calls into question the significance of the organic fraction of diesel exhaust with respect to lung carcinogenesis in the rat. Although a contribution by organic compounds to tumor development cannot be excluded completely, recent evidence emphasizes the highly significant role solid particles play in diesel exhaust's induction of lung cancer in the rat.

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## APPENDIX A. Tumors and Antecedent Lesions in the Lungs of Rodents Exposed to Diesel Exhaust and Carbon Black

### DEFINITIONS OF TUMORS AND OTHER LESIONS

This appendix provides a detailed description of tumors and other lesions associated with development of lung pathology in rodents exposed to diesel exhaust, carbon black, and other particles, along with accepted nomenclature.

The two basic components of both benign and malignant tumors are the parenchyma, made up of transformed or neoplastic cells, and the supporting connective tissue, including vascular structures and, sometimes, lymphatics. The parenchyma of the neoplasm determines to a large extent the neoplasm's biological behavior, and from this component the tumor derives its name. It is recognized, however, that the parenchymal cells could not continue to live and grow without the support of the blood supply and connective tissue components of the proliferating mass.

Types of tumors and other lesions relevant to the studies reported in this paper include the following:

***Benign tumors.*** Benign tumors such as adenomas in the studies referred to in this paper are usually designated by adding the suffix "-oma" to the type of cell from which the tumor derived. For example, a benign tumor arising from cartilage is designated a chondroma; if the tumor arose from fibrous tissue it is called a fibroma. A benign tumor arising from any epithelial surface and exhibiting microscopic or macroscopic fingers or fronds is a papilloma. The term adenoma is used to designate a benign epithelial neoplasm that may or may not exhibit glandlike patterns. In the context of this paper, adenomas of the respiratory system can be described as arising from the bronchiolar-alveolar

epithelium and forming a discrete mass that distorts the normal alveolar or bronchiolar architecture. In this type of tumor, the proliferating cells resemble cuboidal, alveolar, or bronchiolar epithelium and fill the alveolar spaces; the cells may form papillae, pseudoalveoli, or a mixture of these patterns. There is no atypia of individual cells nor is there invasion of the surrounding tissue.

***Malignant tumors.*** The nomenclature used for malignant tumors is similar to that used for benign neoplasms, with some defining additions. Malignant neoplasms that arise in mesenchymal tissues, or derivatives, are called sarcomas. A malignant tumor of fibrous tissue is a fibrosarcoma; a malignant tumor of cartilage is a chondrosarcoma. These are designated by the cell type of which they are composed.

Malignant neoplasms of epithelial origin are designated carcinomas; if they possess a glandular component, or if they are derived from glandular tissue, they are designated adenocarcinomas.

***Adenocarcinomas.*** These malignant neoplasms can be described as a mass of proliferating bronchiolar or alveolar epithelium that form papillae, pseudoalveoli, solid sheets of cells, or a mixture of these patterns. Some of the cells have enlarged, hyperchromatic nuclei, abnormal cell size and shape, or abnormal mitotic figures (atypia). At a point where the tumor joins an area of more normal tissue, there will usually be evidence of invasion. This may not be obvious, however, nor is it essential for the tumor to be categorized as an adenocarcinoma in situ. This in situ designation is assigned on histologic grounds without local invasion or metastasis.

***Squamous cell carcinoma.*** This term denotes a cancer in which the tumor cells resemble stratified squamous epithelium. These cells may derive from stratified squamous epithelium or from epithelium that has undergone squamous metaplasia. This has been described in some of the tumors reported in current studies observed in the bronchiolar-alveolar region of rat lung exposed to diesel exhaust. More specifically, in lung neoplasia a squamous cell carcinoma is a discrete mass of proliferating squamous epithelium originating from metaplastic bronchial, bronchiolar, or alveolar epithelium. There is cellular atypia, lack of polarization of proliferating epithelial cells, and maturation of these cells with attachment to their basement membrane; invasion of adjacent tissues may or may not occur.

A few additional histologic changes observed in the studies reported in this paper are described below because these changes appear to bear on the progressive development (Table 6) from normal to neoplastic cells and on to tumors.

***Hyperplasia, alveolar epithelium.*** This term denotes increased numbers of type II epithelial cells lining the alveoli and alveolar ducts. Normal architecture is preserved but proliferating cells may fill alveolar spaces. Cellular atypia is not part of this type of lesion.

***Hyperplasia, bronchiolar epithelium.*** This term denotes increased numbers of Clara cells lining the lumens of terminal bronchioles; no atypia is observed in this type of lesion.

***Squamous metaplasia, alveolar or bronchiolar epithelium.*** In this type of lesion, normal alveolar or bronchiolar mucous epithelium is replaced with stratified squamous epithelium. Keratinized metaplastic epithelium accumulates in the confined space of the lung and may distort the normal architecture; no atypia is associated with this type of lesion, nor is there abnormal differentiation or polarization with basement membrane.

***Metaplasia, bronchiolar-alveolar.*** This term denotes cases in which the normal epithelium lining the alveoli and alveolar ducts is replaced with Clara cells or ciliated epithelial cells typical of bronchiolar epithelium.

***Squamous cyst (tumor).*** This term denotes a discrete cyst in the lung parenchyma filled with varying amounts of keratinized squamous epithelium. The keratinization of the epithelium is orderly and dysplasia is not observed. Lining epithelium extends into the surrounding alveoli and adjacent bronchioles but does not invade it.

There is a division of opinion among pathologists as to the nature and implication of the squamous cyst (tumor) that is reflected in the variable approaches used to report on the lesion in the series of studies used as a basis for this paper. Some consider the squamous cyst a neoplasm, others do not. Some report the lesion as a squamous tumor without further qualification, but categorize the tumor as benign (Heinrich et al. 1986). Pathologists for some other studies either did not observe such a lesion or did not report it (Iwai et al. 1986). Still others have reported squamous cysts as benign tumors at one point (Mauderly et al. 1987) and later as squamous cysts (nontumors) (Mauderly et al. 1994; Nikula et al. 1995). Classification of the lesion as a squamous cyst (not a tumor), rather than as a benign tumor, seems to be the favored means for many pathologists who record it. It must be recognized, however, that the consensus to classify the lesion as a cyst is a majority opinion, rather than a unanimous one, among pathologists evaluating current animal lung lesions. European pathologists appear to prefer to consider the lesion a neoplasm (International Agency for Research on Cancer 1990, 1992), whereas experimental pathologists in the United States tend to consider it nonneoplastic.

## DIFFERENTIATION BETWEEN TUMORIGENICITY AND CARCINOGENICITY

As noted above, the term tumor denotes cancer in the minds of most individuals. Likewise, the term tumorigenicity is erroneously used interchangeably with carcinogenicity, even in some scientific circles. On a very simple level, a tumorigen is defined as a substance or condition that will induce a tumor. It then follows, by the description of a tumor noted above, that a tumorigen could ostensibly induce cancer. This explanation of a tumorigen is not a correct one, however; in the language of most of those working in the field of oncology, it should not be used to designate a malignancy. The term tumorigen should be reserved for a substance or condition that will induce a benign tumor, not a cancer. Tumorigens and carcinogens, when possible, should clearly denote the appropriate lesion.

There are numerous examples of tumorigens in the literature, most of which are drawn from studies using experimental animals. Perhaps the most extensive listing of substances that produce benign tumors (tumorigens) can be found in the archives and publications of NTP, the United States' premier chemical testing program. For a chemical to be designated as having carcinogenic activity, NTP lists several levels of evidence. The level most related to this discussion is designated as "some evidence" of carcinogenic activity, defined as "demonstrated by studies that are interpreted as showing a chemically related *increased incidence* of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence." Under this scheme of classification a significant number of chemicals that produce only benign tumors are categorized as having "some evidence" for carcinogenic activity and are thus classified as carcinogens.

"Carcinogenicity," on the other hand, strictly applied, is reserved for a substance or condition that induces the development of a cancer (a malignant tumor). Carcinogens produce malignant tumors that, as noted above, will spread and kill the host unless removed or destroyed. One of the better definitions of a carcinogen, and more closely related to this paper, is that proposed almost 30 years ago by Miller and Miller (1966). The description has withstood the test of time during a period of unprecedented growth in cancer research using experimental animals.

> A carcinogen is an agent whose administration to previously untreated animals leads to a statistically significant increased incidence of *malignant* neoplasms as compared with that in appropriate untreated control animals, whether the control animals have low or high spontaneous incidences of the neoplasms in question. Although it would be important to distinguish between agents that produce malignant neoplasms by direct action on the cells that become malignant and those that produce malignancy by indirect actions in animals, at present it is seldom possible to do so. Some agents, including promoters and immune suppressants, can increase the incidence of malignant neoplasms in tissues previously treated with subcarcinogenic doses of carcinogens; such agents should not be termed carcinogens.

The above somewhat lengthy discourse on tumorigens and carcinogens may help to clarify questions that may arise regarding the various types of tumors and other lung lesions reported in relation to diesel exhaust, carbon black, and other particles, as well as how such lesions may relate to causation and implications for human health problems.

## APPENDIX B. Concentrations of Key Constituents of Exposure Atmospheres in a Carbon Black and Diesel Exhaust Tumorigenicity Study

**Table B.1.** Concentrations of Key Constituents of Exposure Atmospheres Collected at Chamber Midpoint During 24 Months[a,b]

| | | Carbon Black | | Diesel Exhaust | | |
|---|---|---|---|---|---|---|
| Constituent | Units | Low Dose | High Dose | Low Dose | High Dose | Control Exposure |
| Total particles | $mg/m^3$ | 2.46 ± 0.03 | 6.55 ± 0.06 | 2.44 ± 0.02 | 6.33 ± 0.04 | 0.05 ± 0.02 |
| Carbon monoxide | ppm | 0.07 ± 0.06 | 0.69 ± 0.06 | 10.30 ± 0.23 | 26.85 ± 0.52 | 0.78 ± 0.06 |
| Carbon dioxide | ppm | 2,010 ± 64 | 1,820 ± 65 | 4,470 ± 77 | 7,390 ± 87 | 2,210 ± 58 |
| Hydrocarbon vapors | ppm | 4.37 ± 0.09 | 4.21 ± 0.08 | 6.47 ± 0.15 | 8.13 ± 0.20 | 4.53 ± 0.08 |
| Total oxides of nitrogen | ppm | 0.030 ± 0.004 | 0.033 ± 0.004 | 8.79 ± 0.32 | 23.45 ± 0.69 | 0.033 ± 0.003 |
| Nitrogen dioxide | ppm | 0.027 ± 0.004 | 0.029 ± 0.004 | 0.73 ± 0.05 | 3.78 ± 0.18 | 0.023 ± 0.002 |

[a] Data are from Mauderly et al. (1994); Nikula et al. (1995).

[b] Values are means ± standard error of weekly mean values for particles and weekly values for vapors and gases. Values for diesel exhaust and carbon black groups presumably include background concentrations of particles, gases, and vapors listed for control exposures.

## APPENDIX C. Estimated Contributions to Tumorigenicity of Diesel Exhaust by Polycyclic Aromatic Hydrocarbons and Nitro–Polycyclic Aromatic Hydrocarbons

As an informative exercise in identifying the major carcinogenic substances in a complex mixture such as diesel exhaust, the simplest approach is to postulate that the carcinogenic risk associated with a specific compound can be expressed as the product of its carcinogenic potency times its concentration in the mixture. Therefore, it is necessary to know the relative potency of the individual compound with respect to the other carcinogens that may be present, as well as the concentration of the compound in the diesel exhaust. There are several experimental limitations in meeting this objective, however: (1) probably a number of significant carcinogens remain chemically unidentified; (2) for the known carcinogens, differences in the animal testing protocols in various laboratories often preclude meaningful comparisons of potencies between compounds; and (3) relatively little information is available on the concentration of many of these carcinogens in diesel exhaust. Thus large gaps remain in our knowledge. At the present time, potency comparisons can be made only among a small number of PAHs and some alkyl- and nitro-PAH derivatives. Furthermore, almost no information is available on the possible synergistic and inhibitory effects presumed to be associated with complex mixtures containing carcinogens, which might influence the simplistic interpretation of carcinogen potency discussed here.

An initial step in such an exercise is to identify animal bioassays that use a consistent experimental protocol, so that meaningful comparisons can be drawn from sources of published information on PAH tumorigenicity. Ideally it would be informative to have inhalation carcinogenicity data for several PAHs to complement the existing inhalation data on diesel exhaust. Aside from the data of Heinrich and associates (1994b) with coal tar/pitch condensation aerosol, however, current long-term inhalation data are insufficient to assess realistically the contribution of PAHs to lung oncogenesis in the rat. Animal bioassays using routes of dosing other than inhalation must be taken into account in the overall evaluation, even though this leaves something to be desired.

Three animal bioassays have been selected for which there are significant data on the carcinogenicity of a number of relevant PAHs and nitro-PAHs: (1) a rat lung implantation assay, (2) a preweanling mouse assay, and (3) a mouse skin-painting assay. Most of these assays were performed within the same laboratory or within different laboratories under nearly identical conditions. In addition, data are available on the tumorigenicity of BaP from all of these assays, thus permitting the calculation of tumorigenic potency relative to BaP for each of the compounds tested.

Table C.1 summarizes the tumorigenic potencies estimated for a number of PAHs and nitro-PAHs that were tested in one or more of the selected animal assays. In general, the compounds have been listed in decreasing order of potency. The potencies are adjusted to molar concentrations and expressed relative to B*a*P (assigned a value of 1.0). Insofar as tumorigenicity and analytical chemistry data are available, this approach estimates the relative contributions of these compounds to the possible carcinogenic activity of any given sample of diesel emissions. Data shown in Table C.1 also allow for a comparison of the potency of a given compound (where data are available) among the different animal bioassays. A number of observations can be made from the information in Table C.1:

1. Many of the well-known PAH carcinogens, B*a*A, the benzofluoranthene series, I*cd*P, and CPP were consistently and substantially less potent than B*a*P.
2. 1-Nitropyrene, one of the major nitro-PAHs in diesel exhaust (International Agency for Research on Cancer 1989a), was negative in all three animal bioassays.
3. Although data are available for only the rat lung implantation assay, 1,6-DNP was 4.5-fold more potent than B*a*P, a finding consistent with its potent carcinogenicity in other animal models (International Agency for Research on Cancer 1989a; Imaida et al. 1991).
4. Indications are that, for the most part, the DBA and DBP isomers were substantially more potent than B*a*P in the preweanling mouse and skin-painting assays.
5. With minor exceptions, agreement between the relative potencies of the various compounds among the three assays was generally good. The major exception is 6-NC, which was a weak tumorigen in the skin-painting assay but highly potent in the preweanling mouse assay. Wislocki and coworkers (1986) have also shown that 6-NC was a potent tumorigen in a one-year preweanling mouse assay with the CD-1 strain, estimated to be nearly twice as potent as B*a*P (relative tumorigenic potency, 1.93).
6. The data presented for the potency of DB*ah*A and chrysene in the skin-painting assay demonstrated good interlaboratory agreement within the limits used to select data for inclusion. For example, the relative potency of 1.73 for DB*ah*A was obtained for a single dose of compound in the CD-1 mouse strain following 26 weeks of TPA promotion (Buening et al. 1979b). The value of 2.12 was estimated from data obtained with 10 subdoses of compound in the SENCAR mouse strain after 20 weeks of TPA promotion (DiGiovanni et al. 1982). Similar examples were found for chrysene.

An application of the estimated tumorigenic potencies of PAHs listed in Table C.1 for the calculation of contributions to tumorigenicity of chemicals in emissions from a variety of light-duty and heavy-duty diesel engines is presented in Tables C.2, C.3, and C.4.

Table C.2 lists results of a potency analysis of the exhaust components in an emissions composite obtained from 6 to 7 four- and five-cylinder light-duty (1.5- to 2.0-L) diesel engines (Volkswagen AG Research and Development 1989). It represents the best sample of light-duty engine emissions data available, in terms of both the number of engines tested and the relative completeness of the chemical analyses performed.

Each compound present in the particle extracts is expressed as its concentration relative to B*a*P on a molar basis and then multiplied by its relative tumorigenic potency in each animal assay from Table C.1 to give a value representing the relative contribution to tumorigenicity. Most of the compounds measured have a tumorigenic impact substantially below that of B*a*P. Among these are unspecified DNPs. Assuming that these DNPs have the same tumorigenic potency as 1,6-DNP (4.53 relative to B*a*P from Table C.1), the total tumorigenic impact is still especially small (0.05) because of the low concentration of DNPs in the extracts.

The single compound that does have a significant tumorigenic contribution is DB*ah*A. It has the same contribution as B*a*P in the skin-painting assay and more than seven times the impact of B*a*P in the preweanling mouse assay. The mixture of DB*ac*A and DB*aj*A isomers, though having only a slight impact by virtue of the low potency of DB*ac*A in the skin-painting assay, is potentially of concern because of the high concentrations of these isomers in the diesel exhaust particle extracts and the lack of information on their potency in other animal bioassays. There is limited evidence for the carcinogenicity of each of the DBA isomers in experimental animals (International Agency for Research on Cancer 1983).

Detailed chemical analyses of the particle extracts from the Nissan and Volkswagen light-duty diesel emissions used in the earlier EPA skin tumor initiation studies (Table C.3) were published by Gallagher et al. (1991). The absolute concentrations of selected PAHs and nitro-PAHs in these emissions, as well as an estimation of the relative tumorigenic contributions for these compounds, are presented in Table C.3. In comparison to the weak tumorigenicity of the Volkswagen extract, the approximately 10-fold increase in numbers of skin tumors observed with the Nissan extract is presumably related to the 20-fold greater concentration of B*a*P and a 4-fold overall increase in other tumorigenic PAHs (Table 11; Table C.3). It is interesting to note that the relatively potent Nissan sample contained only 70% of the 1-NP in the Volkswagen sample and no detectable DNPs. DNPs, however, were present in the relatively inactive Volkswagen sample.

**Table C.1.** Comparative Tumorigenic Potencies of Selected Polycyclic Aromatic Hydrocarbons and Nitro-Polycyclic Aromatic Hydrocarbons Relative to Benzo[*a*]pyrene in Three Animal Bioassays

| | | Potency Relative to Benzo[*a*]pyrene | | | | | |
|---|---|---|---|---|---|---|---|
| Compound | IARC Class[a] | Pre-weanling Mouse Assay | Reference | Lung Implantation Assay | Reference | Skin-Painting Assay | Reference |
| 6-NC | Sufficient | 25.00 | Busby et al. 1988 | — | | 0.02 | El-Bayoumy et al. 1982 |
| 1,6-DNP | Sufficient | — | | 4.53 | Iwagawa et al. 1986 | — | |
| DB*ah*A | Sufficient | 14.49 | Buening et al. 1979b | — | | 1.73<br>2.12 (avg. 1.92) | Buening et al. 1979b<br>DiGiovanni et al. 1982 |
| DB*ac*A | Limited | — | | — | | 0.01 | Slaga et al. 1980 |
| DB*ah*P | Sufficient | >1.0 | Chang et al. 1982 | — | | 4.11 | Chang et al. 1982 |
| DB*ai*P | Sufficient | >1.0 | Chang et al. 1982 | — | | 2.10 | Chang et al. 1982 |
| **B[a]P** | Sufficient | **1.00** | Busby et al. 1984, 1988, 1989; LaVoie et al. 1987 | **1.00** | Deutsch-Wenzel et al. 1983; Iwagawa et al. 1986; Maeda et al. 1986 | **1.00** | Wood et al. 1980; El-Bayoumy et al. 1982; Raveh et al. 1982; DiGiovanni et al. 1982; Rice et al. 1988 |
| B*b*F | Sufficient | 0.21 | LaVoie et al. 1987 | 0.11 | Deutsch-Wenzel et al. 1983 | 0.35 | LaVoie et al. 1982 |
| B*j*F | Sufficient | 0.44 | LaVoie et al. 1987 | 0.03 | Deutsch-Wenzel et al. 1983 | 0.08 | LaVoie et al. 1982 |
| B*k*F | Sufficient | 0.03 | LaVoie et al. 1987 | 0.03 | Deutsch-Wenzel et al. 1983 | 0.02 | LaVoie et al. 1982 |
| CPP | Limited | 0.19 | Busby et al. 1988 | — | | 0.02<br>0.02 | Wood et al. 1980;<br>Raveh et al. 1982 |
| B*a*A | Sufficient | 0.14 | Wislocki et al. 1978 | — | | 0.01 | Wood et al. 1980 |
| Anthanthrene | Limited | — | | 0.19 | Deutsch-Wenzel et al. 1983 | — | |
| Fluoranthene | No | 0.02 | Busby et al. 1984 | — | | — | |
| Chrysene | Limited | 0 | Buening et al. 1979a | — | | 0.04–0.13 (avg. 0.07) | Wood et al. 1980; Slaga et al. 1980; El-Bayoumy et al. 1982; Rice et al. 1988 |
| I*cd*P | Sufficient | 0 | LaVoie et al. 1987 | 0.06 | Deutsch-Wenzel et al. 1983 | 0.01 | Rice et al. 1986 |
| 1-NP | Sufficient | 0 | Busby et al. 1989 | 0 | Maeda et al. 1986 | 0 | El-Bayoumy et al. 1982 |

[a] International Agency for Research on Cancer (Lyon, France) classification of evidence for animal carcinogenicity.

**Table C.2.** Relative Contributions to Tumorigenicity of Polycyclic Aromatic Hydrocarbons and Nitro–Polycyclic Aromatic Hydrocarbons in Light-Duty Diesel Engine Emissions: Volkswagen Data (Particle Extract)[a]

| Compound (μg/mile) | Concentration Relative to Benzo[*a*]pyrene | Comparative Potency Relative to Benzo[*a*]pyrene | | |
|---|---|---|---|---|
| | | Preweanling Mouse (BLU:Ha) Assay | Lung Implantation Assay | Skin-Painting Assay |
| Fluoranthene (112.03) | 14.67 | 0.29 | — | |
| B*a*A (5.44) | 0.71 | 0.10 | — | 0.01 |
| Chrysene (20.82) | 2.73 | 0 | — | 0.19 |
| B*b*F + B*k*F (17.13)[b] | 2.24 | 0.27 | — | 0.40 |
| **BaP (7.64)** | **1.00** | **1.00** | **1.00** | **1.00** |
| I*cd*P (6.32) | 0.83 | 0 | 0.07 | 0.01 |
| Anthanthrene (2.4) | 0.31 | — | 0.06 | — |
| CPP (1.3) | 0.17 | 0.03 | — | 0.003 |
| DB*ah*A (3.9) | 0.51 | 7.39 | — | 0.98 |
| DB*ac*A + DB*aj*A[b] (8.6) | 1.13 | — | — | 0.01[c] |
| 1-NP (5.5) | 0.72 | 0 | 0 | 0 |
| DNPs (0.1)[d] | 0.01 | — | 0.05 | — |
| Total –B*a*P | | 8.08 | 0.18 | 1.60 |

[a] Emissions data are from six to seven light-duty diesel engines (Volkswagen AG Research and Development 1989).

[b] Assuming a 1:1 ratio.

[c] From DB*ac*A only.

[d] Assuming potency of 1,6-DNP (Iwagawa et al. 1986).

**Table C.3.** Relative Contributions to Tumorigenicity of Polycyclic Aromatic Hydrocarbons and Nitro–Polycyclic Aromatic Hydrocarbons in Light-Duty Diesel Engine Emissions: U.S. Environmental Protection Agency Volkswagen Rabbit and Nissan Engine Data (Particle Extract)[a]

| Compound | Concentration (μg/g) | | Concentration Relative to Benzo[*a*]pyrene | | Comparative Potency Relative to Benzo[*a*]pyrene | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Preweanling Mouse (BLU:Ha) Assay | | Lung Implantation Assay | | Skin-Painting Assay | |
| | VW | Nissan | VW | Nissan | VW | Nissan | VW | Nissan | VW | Nissan |
| Fluoranthene | 1,520.0 | 6,120.0 | 50.67 | 10.74 | 1.01 | 0.21 | — | — | — | — |
| B*a*A | 130.0 | 1,030.0 | 4.33 | 1.81 | 0.61 | 0.25 | — | — | 0.04 | 0.02 |
| Chrysene/ Triphenylene[b] | 220.0 | 1,510.0 | 7.33 | 2.65 | 0[b] | 0[b] | — | — | 0.51[b] | 0.19[b] |
| B*b*F | 170.0 | 1,310.0 | 5.67 | 2.30 | 1.19 | 0.48 | 0.62 | 0.25 | 1.98 | 0.80 |
| B*j*F | 90.0 | 740.0 | 3.00 | 1.30 | 1.32 | 0.57 | 0.09 | 0.04 | 0.24 | 0.10 |
| B*k*F | 60.0 | 550.0 | 2.00 | 0.96 | 0.06 | 0.03 | 0.06 | 0.03 | 0.04 | 0.02 |
| **BaP** | **30.0** | **570.0** | **1.00** | **1.00** | **1.00** | **1.00** | **1.00** | **1.00** | **1.00** | **1.00** |
| I*cd*P | 70.0 | 720.0 | 2.33 | 1.26 | — | 0 | 0.19 | 0.10 | 0.02 | 0.01 |
| 1-NP | 589.3 | 406.8 | 19.64 | 0.71 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1,6-DNP | 0.6 | ND[c] | 0.02 | 0 | — | — | 0.09 | — | — | — |
| 1,8-DNP | 0.4 | ND | 0.01 | 0 | — | — | — | — | — | — |
| Total | 2,880 | 12,957 | | | | | | | | |
| Total –B*a*P | | | | | 4.19 | 1.54 | 1.05 | 0.42 | 2.83 | 1.14 |

[a] Emissions data are from Gallagher et al. (1991).

[b] Assuming 100% chrysene.

[c] ND = not detected.