Inhalation Exposure of Rats to Asphalt Fumes Generated at Paving Temperatures Alters Pulmonary Xenobiotic Metabolism Pathways without Lung Injury

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Asphalt fumes are complex mixtures of various organic compounds, including polycyclic aromatic hydrocarbons (PAHs). PAHs require bioactivation by the cytochrome P-450 monoxygenase system to exert toxic/carcinogenic effects. The present study was carried out to characterize the acute pulmonary inflammatory responses and the alterations of pulmonary xenobiotic pathways in rats exposed to asphalt fumes by inhalation. Rats were exposed at various doses and time periods to air or to asphalt fumes generated at paving temperatures. To assess the acute damage and inflammatory responses, differential cell counts, acellular lactate dehydrogenase (LDH) activity, and protein content of bronchoalveolar lavage fluid were determined. Alveolar macrophage (AM) function was assessed by monitoring generation of chemiluminescence and production of tumor necrosis factor-\(\alpha\) and interleukin-1. Alteration of pulmonary xenobiotic pathways was determined by monitoring the protein levels and activities of P-450 isozymes (CYP1A1 and CYP2B1), glutathione-S-transferase (GST), and NADPH:quinone oxidoreductase (QR). The results show that acute asphalt fume exposure did not cause neutrophil infiltration, alter LDH activity or protein content, or affect AM function, suggesting that short-term asphalt fume exposure did not induce acute lung damage or inflammation. However, acute asphalt fume exposure significantly increased the activity and protein level of CYP1A1 whereas it markedly reduced the activity and protein level of CYP2B1 in the lung. The induction of CYP1A1 was localized in nonciliated bronchiolar epithelial (Clara) cells, alveolar septa, and endothelial cells by immunofluorescence microscopy. Cytosolic QR activity was significantly elevated after asphalt fume exposure, whereas GST activity was not affected by the exposure. This induction of CYP1A1 and QR with the concomitant down-regulation of CYP2B1 after asphalt fume exposure could alter PAH metabolism and may lead to potential toxic effects in the lung. Key words: alveolar macrophage, asphalt fumes, CYP1A1, CYP2B1, cytochrome P-450, glutathione S-transferase, lung injury, NADPH:quinone oxidoreductase, pulmonary inflammation, xenobiotic pathways. Environ Health Perspect 111:1215–1221 (2003). doi:10.1289/ehp.5740 available via http://dx.doi.org/ [Online 24 February 2003]

Approximately half a million workers are exposed to asphalt fumes in the United States. The main use of asphalt is for road paving, and the major routes for asphalt fume exposure are pulmonary inhalation and dermal adsorption. Asphalt fumes are complex mixtures of aerosols and vapors that contain various organic compounds, such as aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and heterocyclic compounds containing nitrogen, oxygen, or sulfur (King et al. 1984). Because of the presence of PAHs in the asphalt fumes, the potential exposure to carcinogens is a health concern for exposed workers.

During road-paving operations, the presence of low levels of total PAHs, including benzo(a)pyrene (BaP), in the paving asphalt fumes has been demonstrated through environmental (Monarca et al. 1987) and personal (Watts et al. 1998) monitoring. Exposure of road pavers to these asphalt fume–associated PAHs was supported by the enhanced level of urinary 1-hydroxypyrene (Jarvholm et al. 1999). Some studies have reported a small genotoxic effect in road pavers caused by their occupational exposure to asphalt fumes (Burgaz et al. 1998; Fuchs et al. 1996). In contrast, substantial genotoxic damage was demonstrated in peripheral mononuclear blood cells of roovers (Fuchs et al. 1996). Epidemiologic evaluation showed relatively lower risks for road pavers than for roofers in developing all types of cancers, including lung, stomach, and bladder cancer (Partanen and Boffetta 1994). The difference in cancer risk for pavers and roofers is probably due to the PAH content in the asphalt fumes, which depends on the generating temperature of the fumes. Indeed, paving asphalt fumes are generated at much lower temperatures than are fumes from asphalt roofing applications, and there is a correlation between mutagenic activity and the amount of three- to seven-ring PAHs in these asphalt fumes (Machado et al. 1993). In light of these studies, there is interest in understanding the effects of asphalt fumes on metabolic pathways involved in activation or deactivation of PAHs in the lung.

The carcinogenic effect of PAHs is closely related to the activity of the cytochrome P-450 monoxygenase system. Pulmonary P-450 activities are composites of numerous specific isozymes, including constitutive CYP2B1 and PAH-inducible CYP1A1 (Guengerich et al. 1982). The inducible CYP1A1 can metabolize PAHs to form carcinogenic metabolites, which may lead to DNA damage (Burke et al. 1985; Golden et al. 1987; Lacy et al. 1992). Repression of CYP2B1 has been demonstrated in some pneumotoxin-induced lung injuries—for example, in response to 1-nitrosothiophene or ipomeanol (Verschoyle and Dinsdale 1990; Verschoyle et al. 1993). However, this toxin-induced injury was not affected by the induction of CYP1A1, suggesting that alteration of CYP2B1 may result in a pulmonary-specific toxic response. In addition to phase I P-450 systems, various phase II enzymes, including glutathione S-transferases (GSTs) and NADPH:quinone oxidoreductase (QR), play important roles in xenobiotic metabolism (Jaiswal 1994; Prestera et al. 1993). Both the induction of CYP1A1 and the lack of GST have been demonstrated to play a role in cancer susceptibility of smokers and coke-oven workers (Bartsch et al. 1999). On the other hand, QR is known to be able to reduce the binding of quinone to DNA and proteins, thus offering a protective effect against genotoxicity (Joseph and Jaiswal 1994). Because the lung is the major target organ for the airborne environmental toxins, exposure to such agents may significantly modulate metabolic pathways and lead to toxic/carcinogenic effects.

A recent epidemiologic study has reported that asphalt fume exposure did not cause significant changes in lung function or pulmonary symptoms among road pavers (Gamble et al. 1999). Previous studies carried out in our laboratory have shown that exposure of rats by intratracheal instillation to paving-temperature asphalt fume condensate, collected at the top of an asphalt storage tank using a cold trap, did not cause lung inflammation or cellular damage and did not alter macrophage function (Ma et al. 2000). However, intratracheal exposure to asphalt condensate significantly induced CYP1A1 in the lung (Ma et al. 2002). These studies show that the major pulmonary effects of asphalt fumes, which lack a particulate component, are not on macrophage-mediated...
inflammatory responses but are on the metabolic enzyme systems that are critical in handling inhaled chemical substances. However, levels of asphalt fume condensate administered by intratracheal instillation were very high, and the acute effects of asphalt fumes on the lung after inhalation exposure have not been investigated. One critical factor in designing an asphalt exposure study is to carry out the inhalation exposure using asphalt fumes generated under road-paving conditions. Such a system would allow characterization of both the inflammatory effects and the modulation of the metabolic enzyme systems resulting from paving asphalt fume exposure. For this reason, an inhalation exposure system has been developed and characterized in our laboratory (Wang et al. 2001).

At present time, a threshold limit value (TLV) for asphalt fumes of 0.5 mg/m³ (8-hr time-weighted average) is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH 2002). The current National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) for asphalt fume exposure is 5 mg/m³ as a ceiling value, which is the limit that would allow characterization of both the inflammatory effects and the modulation of the metabolic enzyme systems resulting from paving asphalt fume exposure. For this reason, an inhalation exposure system has been developed and characterized in our laboratory (Wang et al. 2001).

At present time, a threshold limit value (TLV) for asphalt fumes of 0.5 mg/m³ (8-hr time-weighted average) is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH 2002). The current National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) for asphalt fume exposure is 5 mg/m³ as a ceiling value, which is a concentration not to be exceeded at any time during the workday (NIOSH 2002). However, a permissible exposure limit for asphalt fumes has not yet been promulgated by the Occupational Safety and Health Administration. Because both ACGIH TLV and NIOSH REL values are simply recommendations, workplace exposures to asphalt fumes may exceed these levels and may be of occupational concern. In the present study, we report the effects of inhaled asphalt fumes generated at paving temperatures on pulmonary damage and inflammation and on the metabolic pathways of the lung involving both phase I and phase II enzymes.

Materials and Methods

Asphalt inhalation exposure system. The asphalt fume generation and inhalation exposure systems have been described previously (Wang et al. 2001). Briefly, road paving asphalt was preheated to 170°C in an oven, transferred to a reservoir (at 170°C), and passed through a heated pipe and onto a heated plate through a heated pipe and onto a heated plate. The asphalt fume condensate tube (SKC Inc., Eighty Four, PA) and a charcoal sorbent tube to collect medium- and small-molecular-weight chemicals, and these samples were used for chemical analysis of the fume generator output as described previously (Wang et al. 2001). The filters were weighed immediately after the sampling period ended, and the fume concentration was determined. In the present study, rats were exposed to asphalt fumes generated at paving temperatures for various exposure time periods. The calculated total asphalt fume exposure (milligrams per hour per cubic meter) for each treatment group (Table 1) was calculated as the product of the asphalt fume concentration (milligrams per cubic meter) and the total exposure time (hours per day × days).

Treatment of animals. Female Sprague-Dawley rats [H/SDC(CVF) from Hilltop Lab Animals (Scottsdale, PA), monitored free of endogenous viral pathogens, parasites, mycoplasmas, Helicobacter, and cilia-associated respiratory bacillus and weighing about 150 g (~5 weeks old) at arrival, were used for all experiments. The rats were kept in cages ventilated with filtered air on Alpha-dri virgin cellulose chips (ALPHA-dri, Shepherd Specialty Papers; T.R. Last Company, Gibsonia, PA) and hardwood Beta-chips (Beta Chip, Northeastern Products Corp.; Famers Delight Company, Grafton, WV) as bedding and were provided filtered air on XAD-2 sorbent and charcoal sorbent tube to collect medium- and small-molecular-weight chemicals, and these samples were used for chemical analysis of the fume generator output as described previously (Wang et al. 2001). The filters were weighed immediately after the sampling period ended, and the fume concentration was determined. In the present study, rats were exposed to asphalt fumes generated at paving temperatures for various exposure time periods. The calculated total asphalt fume exposure (milligrams per hour per cubic meter) for each treatment group (Table 1) was calculated as the product of the asphalt fume concentration (milligrams per cubic meter) and the total exposure time (hours per day × days).

Table 1. Total asphalt fume exposure.

| Exposure concentration (mg/m³) | Exposure time | Total exposure concentration (mg hr/m³) |
|-------------------------------|---------------|--------------------------------------|
| 52.9                         | 1 hr          | 52.9                                 |
| 58.0 ± 5.6                   | 6 hr          | 362.9 ± 33.6                         |
| 10.4 ± 1.4                   | 3.5 hr/day, 5 days | 182 ± 24.5                          |
| 23.6 ± 3.3                   | 3.5 hr/day, 5 days | 413 ± 57.7                          |
| 16.7 ± 1.9                   | 6 hr/day, 5 days | 547 ± 57.9                          |
| 24.4 ± 2.0                   | 6 hr/day, 5 days | 732 ± 60                            |
| 57.8 ± 3.0                   | 6 hr/day, 5 days | 1,734 ± 90                           |

| Protein, LDH activity, and chemiluminescence determination. The acellular LDH activity in BALF was monitored and used as a cytotoxicity index. LDH activity was determined by measuring the formation of NADH, which was monitored spectrophotometrically at 340 nm, using Roche Diagnostic Systems (Indianapolis, IN) reagents and procedures, on an automated Cobas FARA II analyzer (Roche Diagnostic Systems, Montclair, NJ). The protein content in the acellular BALF, used as a marker for air–blood barrier damage, was measured using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL) following the manufacturer’s protocol.

Chemiluminescence (CL) generated by AMs was determined using an automated luminometer (Berthold Autolumat LB 953, Wallac, Inc., Gaithersburg, MD). Rat AMs (1 × 10⁶/mL) were preincubated at 37°C for 10 min in HEPES-buffered medium, containing 0.08 µg/mL luminol. Zymosan (2 mg/mL) was added to the preincubated AM sample, and
The activities of CYP2B1 and CYP1A1 were determined by measuring the O-dealkylation of 7-pentoxysresorufin (PROD) and 7-ethoxysresorufin (EROD), respectively, in microsomal suspensions, containing 0.1 mg/mL microsomal protein in an incubation medium buffered at pH 7.6 according to the method of Burke et al. (1985). The reaction mixture contained 5 µM 7-pentoxysresorufin or 3 µM 7-ethoxysresorufin (Sigma Chemical Co., St. Louis, MO) and was initiated by addition of 0.48 mM NADPH at 37°C. The activities of PROD and EROD were measured by monitoring the formation of resorufin spectrophotometrically at an excitation wavelength of 530 nm and emission of 585 nm (model LS-50 Luminescence Spectrometer; Perkin-Elmer Corp., Norwalk, CT) at various times. The results were expressed as picomoles of resorufin formed per minute per milligram of microsomal protein.

GSTs are a group of enzymes catalyzing the conjugation of reduced glutathione (GSH) with a wide variety of electrophilic compounds. GST activity was determined using the generic GST substrate 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma) according to the method of Habig et al. (1974). The final reaction mixture contained 1 mM CDNB and 1 mM GSH in 0.1 M potassium phosphate buffer (pH 6.5). The reaction was started by the addition of lung-soluble supernate (cytosol), and the increase in absorbance was measured spectrophotometrically at 340 nm using a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Columbia, MD).

The cytosolic QR activity was determined by monitoring dicumarol-inhibitable QR activity, using 2,6-dichlorophenolindophenol (Sigma Chemical Co., final concentration 40 µM) as the substrate (Benson et al. 1980). The loss of optical density at 600 nm was recorded for 10 min at 37°C using a Shimadzu Spectrophotometer; Perkin-Elmer Corp., Norwalk, CT) followed by a 2 hr incubation at 37°C. The activities of PROD and EROD were determined by measuring the formation of resorufin spectrophotometrically at an excitation wavelength of 530 nm and emission of 585 nm (model LS-50 Luminescence Spectrometer; Perkin-Elmer Corp., Norwalk, CT) at various times. The results were expressed as picomoles of resorufin formed per minute per milligram of microsomal protein.
an index of inflammation. Protein content and LDH activity in acellular first lavage fluid were measured to monitor injury to the air–blood barrier and cell cytotoxicity, respectively. Table 2 shows that acute exposure of rats to asphalt fumes by inhalation did not cause significant neutrophil infiltration into the air space. The amount of total protein and the activity of LDH in the acellular lavage fluid from asphalt fume–exposed rats were also normal compared with the controls. These results suggest that short-term exposure of rats to paving-temperature asphalt fumes did not cause significant pulmonary inflammation or lung injury.

Acute effects on AM activity. Table 3 shows the effects of asphalt fume exposure on the production of reactive oxygen species and proinflammatory cytokines by AMs. The respiratory burst activity of AMs, indicated by the cellular generation of CL in response to zymosan stimulation, and the production of proinflammatory cytokines TNF-α and IL-1 by AMs with or without lipopolysaccharide (LPS) stimulation, were not significantly affected by acute asphalt fume exposure. The results shown in Tables 2 and 3 confirm our earlier intratracheal instillation findings that asphalt condensate collected from fumes generated at road-paving temperatures did not cause damage to the air–blood barrier or cytotoxicity in the lung and did not activate or depress AM function measured as secretion of proinflammatory cytokines or other reactive species (Ma et al. 2000).

Microsomal protein content and cytochrome P-450 reductase activity in lung microsomes. Table 4 shows the effects of asphalt fume inhalation on the microsomal protein content and the NADPH cytochrome c reductase activity of the lung. The results indicate that inhalation of asphalt fumes generated at paving temperatures did not significantly alter the total protein level or the NADPH cytochrome c reductase activity of lung microsomes.

Alteration of CYP1A1 and CYP2B1 protein levels and activities. The CYP1A1 and CYP2B1 activities in lung microsomes were determined by monitoring the dealkylation of EROD or PROD, respectively. Figure 1 shows the biotransformation of 7-ethoxyresorufin (Figure 1A) and 7-pentoxysresorufin (Figure 1B) to resorufin in microsomal suspensions from rats exposed to clean air or to asphalt fumes generated at paving temperatures. The results show that asphalt fume exposure induced microsomal CYP1A1–specific EROD activity compared with air-exposed controls in a total exposure-dependent manner. In contrast, microsomal CYP2B1–specific PROD activity was progressively reduced as the total asphalt fume exposure increased.

Table 2. The effects of asphalt fume exposure on pulmonary inflammation and lung injury.

| Treatment | PMNs (x10⁶) | LDH (U/L) | Protein (mg/mL) |
|-----------|-------------|-----------|----------------|
| 3.5 hr/day, 5 days | | | |
| Air control | 1.04 ± 0.15 | 49 ± 5 | 0.57 ± 0.06 |
| Asphalt (414 mg hr/m³) | 0.79 ± 0.05 | 56 ± 5 | 0.50 ± 0.04 |
| 6 hr/day, 5 days | | | |
| Air control | 0.72 ± 0.08 | 49 ± 4 | 0.37 ± 0.04 |
| Asphalt (547 mg hr/m³) | 0.79 ± 0.06 | 60 ± 5 | 0.40 ± 0.05 |
| 3.5 hr/day, 5 days | | | |
| Air control | 1.22 ± 0.16 | 85 ± 4 | 0.43 ± 0.01 |
| Asphalt (1,733 mg hr/m³) | 0.71 ± 0.07 | 83 ± 11 | 0.42 ± 0.03 |

Rats were exposed to air or asphalt fumes for 5 days, at total asphalt exposures of 414 mg hr/m³, 547 mg hr/m³, or 1,733 mg hr/m³, and sacrificed the next day. Values represent mean ± SE (n = 8).

Table 3. Effects of paving asphalt fume exposure on AM function.

| Treatment | CL (10⁵ counts/min/10⁶ cells) | TNF-α (ng/10⁶ AMs) | IL-1 (dpn/10⁶ AMs) |
|-----------|-------------------------------|-------------------|-------------------|
|           | Resting | Zymosan stimulated | LPS | LPS | Resting | LPS | LPS |
| 3.5 hr/day, 5 days | | | | | | | |
| Air control | ND | ND | 388 ± 83 | 15,038 ± 3,153 | 906 ± 157 | 84,504 ± 11,878 |
| Asphalt (182 mg hr/m³) | 3.41 ± 0.06 | 0.91 ± 0.12 | 382 ± 134 | 12,522 ± 2,487 | 851 ± 68 | 60,798 ± 12,091 |
| 3.5 hr/day, 5 days | | | | | | | |
| Air control | 1.41 ± 0.05 | 1.21 ± 0.07 | ND | ND | ND | ND |
| Asphalt (414 mg hr/m³) | 1.38 ± 0.05 | 1.21 ± 0.07 | ND | ND | ND | ND |
| 6 hr/day, 5 days | | | | | | | |
| Air control | 1.45 ± 0.05 | 0.89 ± 0.14 | 398 ± 61 | 1,501 ± 1,578 | 1,756 ± 168 | 3,509 ± 905 |
| Asphalt (547 mg hr/m³) | 1.60 ± 0.08 | 2.02 ± 0.43 | 740 ± 187 | 5,162 ± 1,993 | 1,550 ± 201 | 2,529 ± 496 |
| 6 hr/day, 5 days | | | | | | | |
| Air control | ND | ND | 1,507 ± 340 | ND | 2,011 ± 243 | ND |
| Asphalt (1,733 mg hr/m³) | 1,733 mg/hr/m³ | ND | 2,372 ± 1,111 | 2,080 ± 378 | ND |

ND, not determined. The resting and zymosan-stimulated CL generated by AMs was determined to monitor AM respiratory burst activity. TNF-α and IL-1 production by AMs with or without LPS (0.1 µg/10⁶ cells) stimulation was assayed to monitor the effects of asphalt fume exposure on macrophage secretion of proinflammatory cytokines. Values represent mean ± SE (n = 6–8).

The CYP1A1 and CYP2B1 proteins were fractionated by SDS/PAGE and transferred to a nitrocellulose membrane for Western blot analysis using rabbit polyclonal antibodies specific for CYP1A1 and CYP2B1. As shown in Figure 2A, there was very little CYP1A1 antibody–reactive protein in microsomes obtained from control lungs. However, after asphalt fume exposure, there was a marked induction of immunoreactive CYP1A1 protein in the rat lung microsomes. An increase of total asphalt fume exposure from 53 to 1,733 mg hr/m³ produced an exposure-dependent induction of CYP1A1 protein in lung microsomes (Figure 2B). The rat lung microsomes have relatively high endogenous levels of CYP2B1, as shown in Figure 2C. Figure 2D shows that the constitutive CYP2B1 content was not affected by the asphalt fume exposure at total exposures of 53 and 353 mg hr/m³ but was significantly attenuated at asphalt fume exposures of 414 mg hr/m³ or higher. This asphalt fume concentration-dependent reduction of CYP2B1 in lung microsomes was found to correlate with the inhibitory effects of asphalt fume exposure on PROD activity (Figure 1).

Localization of CYP1A1 in asphalt fume–exposed lungs. Figure 3 shows the localization of CYP1A1 in asphalt fume (547 ± 57.9 mg hr/m³)–exposed rat lung tissue using an immunofluorescence technique. After exposure to asphalt fumes, CYP1A1 is induced and is localized mainly in alveolar septa, nonciliated airway epithelial cells, and endothelial cells, as indicated by the green fluorescence.

GST and QR activity. The effects of asphalt fume exposure on phase II drug metabolism enzymes GST and QR were monitored in the lung cytosolic fraction. Exposure

Table 4. Microsomal protein content (mg/g lung) and NADPH cytochrome c reductase activity (nmol/min/mg of microsomal protein) microsomes isolated from air control and paving asphalt fume–exposed rats.

| Treatment | Microsomal protein | NADPH cytochrome c reductase activity |
|-----------|--------------------|-------------------------------------|
| 3.5 hr/day, 5 days | | |
| Air control | 4.38 ± 0.29 | ND |
| Asphalt (414 mg hr/m³) | 4.19 ± 0.20 | |
| 6 hr/day, 5 days | | |
| Air control | 3.07 ± 0.18 | 14.15 ± 1.06 |
| Asphalt (547 mg hr/m³) | 3.26 ± 0.19 | 12.68 ± 0.60 |
| 6 hr/day, 5 days | | |
| Air control | 4.11 ± 0.41 | 20.29 ± 4.32 |
| Asphalt (1,733 mg hr/m³) | 4.11 ± 0.23 | 18.95 ± 0.42 |

ND, not determined. Microsomes were isolated from air-exposed or asphalt fume–exposed lungs by differential centrifugation, and microsomal protein content was determined by protein assay using a bicinchoninic acid method. Results were normalized to microsomal protein content and are presented as the mean ± SE (n = 8).
of rats to asphalt fumes generated at paving temperatures did not alter the total cytosolic GST activity, as shown in Table 5, or the protein level of GST-α (data not shown). In contrast, except at the low total asphalt fume concentration of 53 mg hr/m³, asphalt fume exposure significantly induced cytosolic QR activity compared with air-exposed controls (Table 5).

Discussion

Pulmonary tissue is a major target for various environmental airborne pollutants and chemicals. This study shows that inhalation exposure of rats to asphalt fumes generated at paving temperatures does not cause acute damage to the alveolar air–blood barrier, cytokotoxicity, or inflammation, as indicated by acellular BALF levels of protein and LDH activity, PMN infiltration, and CL generation and cytokine production by AMs. In addition, asphalt fume inhalation did not compromise the ability of AMs to respond to zymosan or bacterial LPS ex vivo. However, a clear modulation of the pulmonary xenobiotic metabolic pathways was observed in response to acute asphalt fume exposure. Acute asphalt fume exposure produced a chemical effect that altered both phase I and phase II enzyme levels and activities in the lung, including the induction of CYP1A1 and QR and a concomitant reduction of CYP2B1 without affecting GST activity. This alteration of xenobiotic enzymes in the lung may significantly affect PAH metabolism and lead to increased susceptibility of the lung to toxic effects.

Reports concerning the direct pulmonary effects of asphalt fume exposure on road workers are not consistent. Studies have shown that asphalt fume exposure induced subjective symptoms (Norseth et al. 1991) and pulmonary disorders in some pavers (Maizlish et al. 1988; Norseth et al. 1991); however, Gamble et al. (1999) recently reported no consistent association between asphalt fume exposure level and the reduction in lung function or the incidence of symptoms among road workers. The present study shows that exposure of rats to asphalt fumes generated at paving temperatures did not induce infiltration of neutrophils into the air spaces or cause damage to the air–blood barrier or cytotoxicity.

In addition, asphalt fume exposure did not activate AMs to generate reactive oxygen species or produce inflammatory cytokines TNF-α and IL-1. Furthermore, it did not significantly affect the ability of AMs to respond to microbial products, measured as oxidant production in response to zymosan or cytokine production in response to LPS. These results are consistent with those of a previous study indicating that exposure of rats by intratracheal instillation with asphalt fume condensate, collected from the top of an asphalt storage tank, did not cause pulmonary damage, induce inflammation, or alter AM activity (Ma et al. 2000). This lack of acute pulmonary responses to asphalt fume exposure suggests that asphalt fumes are probably not processed by AMs in the same manner as are organic compounds adsorbed on particles, for example, diesel exhaust particles (DEP). Indeed, intratracheal instillation of rats with DEP particles, with adsorbed organic compounds, caused lung injury and inflammation, increased basal oxidant and cytokine production, but depressed the ability of AMs to generate oxidant species or produce cytokines in response to subsequent zymosan or LPS treatment (Castranova et al. 2001; Yang et al. 1997, 1999).

Investigation of the potential carcinogenic and/or mutagenic effects of asphalt fume exposure during road paving have yielded inconsistent results. However, roofers exposed to asphalt fumes generated at higher temperatures have consistently exhibited a greater risk for developing lung and stomach cancers than did road pavers (Partanen and Boffetta 1994) and exhibit substantial genotoxic damage in peripheral mononucleoed blood cells (Fuchs et al. 1996). Therefore, one might ask whether relatively high-molecular–weight PAHs (i.e., those that might present a cancer risk) are generated at paving temperatures. Chemical analysis of asphalt fumes, produced by our generator, has demonstrated the presence of PAHs (Wang et al. 2001). The present study shows that exposure of rats to asphalt fumes generated at paving temperatures resulted in inhaled PAH levels that were sufficient to alter pulmonary xenobiotic metabolic enzymes, causing induction of CYP1A1 as well as reduction of CYP2B1. However, our previous study demonstrated that exposure of rats by intratracheal instillation to asphalt fume condensate induced pulmonary CYP1A1 without affecting the constitutive CYP2B1 (Ma et al. 2002). The discrepancies between these two studies may be caused by the exposure route and the chemical composition of the asphalt fume—that is, fume condensate versus the whole fume. Because PAHs are the inducers as well as substrates for CYP1A1, the chemical composition of the fumes may play an important role in the
modification of xenobiotic metabolism in the exposed lung. The chemicals contained in the whole fume generated in the laboratory included not only the higher-boiling-point compounds but also the smaller compounds formed in the vapor phase; however, the cold-trap-collected fume condensate may not contain some of the compounds in the vapor phase of the whole fume that may be capable of modulating the metabolic enzymes. This is possible because exposure of mice to naphthalene, a small-molecular–weight component of asphalt fumes, can significantly reduce pulmonary P-450 content and microsomal enzyme activities (Tong et al. 1982). Decreased CYP2B1 has also been shown in the rabbit lung after β-naphthoflavone (β-NF) treatment (Bhagwat et al. 1999) but not in 3-methylcholanthrene (3MC)-exposed rat lungs (Gozukara et al. 1982), suggesting that alteration of P-450 metabolic pathways is especially substrate dependent. In this light, any change in the fume composition may result in a significant difference in its ability to modify metabolic pathways.

The PAH-mediated increase of CYP1A1 by asphalt fumes is in good agreement with the effects of exposure to other mixed exposure systems, including cigarette smoke (Willey et al. 1997) and DEP (Pott and Heinrich 1990). Cytochrome P-450 isoenzymes are known for their substrate selectivity. In the case of BaP, CYP1A1 prefers bay-region (7,8 positions) oxidation (Gozukara et al. 1982) and yields metabolites that can be further metabolized to ultimate carcinogens, whereas CYP2B1 catalyzes oxidation of BaP at the 4,5 positions (Gozukara et al. 1982) to yield metabolites that can be easily removed (Smith and Bend 1980). This demonstrates that different isoforms are involved in the regulation of the balance between activation and detoxification pathways of PAH metabolism. Therefore, induction of CYP1A1 with down-regulation of CYP2B1 after asphalt fume exposure may lead to increased production of toxic metabolites of BaP.

There are more than 40 different cell types in the lung. Asphalt fume-induced CYP1A1, as shown by immunofluorescence microscopy, was localized mainly in nonciliated bronchiolar epithelial (Clara) cells, the alveolar septa, and endothelial cells. We found no evidence of proliferation of any specific cell type, but instead observed site-specific induction localized to these areas. These results are consistent with the findings for 3MC-exposed (Keith et al. 1987) or β-NF–exposed rat lungs (Lacy et al. 1992). The localization of CYP1A1 in selected lung cell populations suggests that there may be selective targets in the lung due to metabolic activation of toxic compounds in certain individual cell types.

Asphalt fume exposure did not alter GST but enhanced the QR activity in the lung. These phase II enzymes are known for their detoxification activities. Using cells transfected with plasmids that express elevated levels of selective enzymes individually or in combination, Joseph and Jaiswal (1994) demonstrated that semiquinone, a metabolite of BaP generated after CYP1A1 and NADPH P-450 reductase activation, resulted in distinct BaP–DNA adducts. However, the formation of DNA adducts was significantly reduced by inclusion of a high level of QR activity. These studies clearly demonstrate the importance of keeping these enzymes in balance, and the important role of phase II enzymes in PAH–induced DNA damage. In addition, there is an interactive effect resulting from exposure to mixed PAHs. Pott and Heinrich (1990) have shown in animal studies that inhaled cigarette smoke or DEP contains much less BaP than do fumes from a coke oven or tar pitch but exhibits similar carcinogenic activity. These studies suggest that exposure to mixed PAHs

Table 5. GST and QR activities in cytosolic proteins isolated from air control and paving asphalt fume–exposed rats.

| Treatment | GST activity (nmol/min/mg protein) | QR activity (nmol/min/mg protein) |
|-----------|-----------------------------------|----------------------------------|
| 1 hr      |                                   |                                  |
| Air control | 184 ± 16                          | 742 ± 39                         |
| Asphalt (52.9 mg hr/m3) | 143 ± 8                            | 764 ± 24                          |
| 3.5 hr/day, 5 days | ND | 439 ± 39                            |
| Air control |                                   | 836 ± 28*                          |
| Asphalt (414 mg hr/m3) |                                   |                                  |
| 6 hr/day, 5 days |                                   |                                  |
| Air control | 369 ± 31                           | 783 ± 28                          |
| Asphalt (732 mg hr/m3) | 363 ± 37                           | 1,291 ± 32*                        |
| 6 hr/day, 5 days |                                   |                                  |
| Air control | ND | 592 ± 91                            |
| Asphalt (1,733 mg hr/m3) | 1,375 ± 292*                        |

ND, not determined. Cytosolic proteins were isolated from air- or asphalt fume–exposed lungs by differential centrifugation, and cytosol protein content was determined. GST activity was determined by measuring the binding of GSH with CDNB. The QR activity was monitored by measuring the reduction of 2,6-dichlorophenolindophenol by cytosolic protein. Values represent means ± SE (n = 4).

*Significantly different from air control group, p < 0.05.

Figure 3. Immunofluorescence micrographs of CYP1A1 localization in air-exposed and asphalt fume–exposed (547 mg hr/m3) rat lungs. CYP1A1 was labeled with a green fluorochrome (Alexa 488). Green fluorescence indicates the localization of CYP1A1. (A) Airways of control rats. (B) Airways of control rats. (C) Airways of asphalt-exposed rats. (D) Airways of asphalt-exposed rats. Arrows point to sites of immunoreactivity. Bars = 20 μm.
may synergistically induce carcinogenic effects. Boffetta et al. (1997) have shown that genetic cancer susceptibility and DNA adducts in smokers, tobacco chewers, and coke oven workers are related to the up-regulation of a cancer-predisposing gene, CYP1A1, and lack of a protective factor, GSTM1.

In summary, the present study shows that acute exposure to asphalt fumes generated in the laboratory under road-paving temperatures did not cause alveolar capillary damage or pulmonary inflammation or alter AM activity. However, such exposure significantly altered xenobiotic metabolizing enzymes in the lung, characterized as the induction of CYP1A1 and QR and a down-regulation of CYP2B1. Such alteration in metabolic enzymes would favor PAH activation for the formation of toxic PAH metabolites that might lead to mutagenic/carcinogenic effects in the lung.

**REFERENCES**

ACGIH. 2002. TLVs and BEIs: Threshold Limit Values for Chemicals Substances and Physical Agents; Biological Exposure Indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Bartsch H, Rojas M, Nair U, Nair J, Alexandrov K. 1989. Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. Cancer Detect Prev 23:449–452.

Benson AM, Hunkele MJ, Talalay P. 1980. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc Natl Acad Sci USA 77:5216–5220.

Boffetta P, Jurenkova N, Gustavsson P. 1997. Cancer risk from asphalt and tobacco smoke in photographer and printer workers. Occupational and Environmental Exposure. Memphis, TN: National Cotton Council, 131–135.

Castranova V, Jones TA, Barger MW, Afshari A, Frazer DG. 1989. Pulmonary responses of guinea pigs to consecutive exposures to cotton dust. In: Proceedings of the 14th Cotton Dust Research Conference. Jacobs RR, Wakeley PJ, Dometalsh LN, eds. Memphis, TN: National Cotton Council, 131–135.

Castranova V, Ma JY, Young HM, Antonini JM, Butterworth L, Barger MW, et al. 2001. Effect of exposure to diesel exhaust particles on the susceptibility of the lung to infection. Environ Health Perspect 109(suppl 4):609–612.

Fuchs J, Hengstler JG, Bottiger G, Desch F. 1996. Primary DNA damage in peripheral mononuclear blood cells of workers exposed to bitumen-based products. Int Arch Occup Environ Health 68:141–146.

Gamble JF, Nicolich MJ, Barone NJ, Vincent WJ. 1999. Exposure-response of asphalt fumes with changes in pulmonary function and symptoms. Scand J Work Environ Health 25:196–206.

Goddin PM, Kass G, Mayer RT, Burke MD. 1987. The effects of cigarette smoke compared to 3-methylcholanthrene and phenobarbitone on alkoyoxyresorufin metabolism by lung and liver microsomes from rats. Biochem Pharmacol 36:3393–3398.

Gouzarka EM, Guengerich FP, Miller H, Gelboin HV. 1982. Different patterns of benzo(a)pyrene metabolism of purified cytochromes P-450 from methylcholanthrene, beta-naphthoflavone and phenobarbital treated rats. Carcinogenesis 3:129–133.

Guengerich FP, Wang P, Davidson NK. 1982. Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and humans using immunochromatographic coupling with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biochemistry 21:1699–1708.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139.

Jaishwal AK. 1994. Antioxidant response element. Biochem Pharmacol 48:439–444.

Jarvholm B, Nordstrom G, Hogstedt B, Levin JO, Wahlstrom J, Ostman C, et al. 1999. Exposure to polycyclic aromatic hydrocarbons and genotoxic effects on nonsmoking Swedish road pavement workers. Scand J Work Environ Health 25:131–136.

Joseph P, Jaishwal AK. 1994. NAD(P)H:quinone reductase (1) diisopropyl phosphate-stabilized form of rat and mouse liver microsomes: purification and properties. Toxicol Appl Pharmacol 156:231–240.

Keith IM, Olson EBJ, Wilson NM, Jefcoate CR. 1987. Cytosolic glutathione-associated enzyme activities in freshly isolated alveolar macrophages and lung tissues of smokers and nonsmokers. Exp Lung Res 3:129–133.

Khan JH, Lewis DM, Castranova V, Rojinasakul Y, Banks DE, Ma JY, et al. 1992. Inhibitory action of tartaric acid on macrophage production of interleukin-1 (IL-1)–like activity and thymocyte proliferation. Exp Lung Res 18:715–729.

Krug IM, Dixon EB, Wilson NM, Jefcoate CR. 1987. Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450. Cancer Res 47:1878–1882.

Keith IM, Dixon EB, Wilson NM, Jefcoate CR. 1987. Immunological identification and effects of 3-methylcholanthrene and phenobarbital on rat pulmonary cytochrome P-450. Cancer Res 47:1878–1882.

King RW, Puzinauskas VP, Holdsworth CE. 1984. Asphalt fume condensate exposure. J Toxicol Environ Health 5:179–188.

Lacy SA, Mangum JB, Everitt JI. 1992. Cytochrome P-450- and rho-protein expression in rat lung. Lung 171:114–124.

Miles PR, Ma JY, Bowman L, Miller MR. 1996. Pulmonary microsomal metabolism of benzo(a)pyrene following exposure of rats to silica. J Toxicol Environ Health 48:501–514.

Monarca S, Pasquini R, Scassellati SG, Savino A, Bauleo FA, Angeli G. 1987. Environmental monitoring of mutagenic/carcinogenic hazards during road paving operations with bitumens. Int Arch Occup Environ Health 59:393–402.

NIOSH. 2002. NIOSH Pocket Guide to Chemical Hazards and Other Databases. NIOSH Publication No. 2002-140. CD-ROM. Cincinnati, OH: National Institute for Occupational Safety and Health.

Norseth R, Waage IA, Waller L. 1991. Acute effects and exposure to organic compounds in road maintenance workers exposed to asphalt. Am J Ind Med 20:737–744.

Partanen T, Boffetta P. 1994. Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiological studies. Am J Ind Med 26:721–740.

Patt F, Heinrich U. 1990. Relative significance of different hydrocarbons for the carcinogenic potency of emissions from various incomplete combustion processes. IARC Sci Publ 10:288–297.

Prestera T, Zhang Y, Spencer SR, Wilczak CA, Talalay P. 1993. The electrophilic counterattack response: protection against neoplasia and toxicity. Adv Enzyme Regul 33:281–296.

Smith BR, Bend JR. 1980. Prediction of pulmonary benzo(a)pyrene 4,5-dioleic cleavage: a pharmacokinetic analysis of epoxide-metabolizing enzymes in rabbit lung. J Pharmacol Exp Ther 241:478–482.

Sprung SS, Loeve MC, Trush MA, MMAughr G, Ginsburg E, Hirokata Y, et al. 1982. Bronchiolar epithelial damage and impairment of pulmonary microsomal monoxygenase activity in mice by naphthalene. Exp Mol Pathol 37:358–369.

Verschoyle RD, Dinsdale D. 1990. Protection against chemical-induced lung injury by inhibition of pulmonary cytochrome P-450. Environ Health Perspect 85:95–100.

Verschoyle RD, Dinsdale D, Wolf CR. 1990. Inhibition and induction of cytochrome P450 isoenzymes in rat lung. Toxicol Appl Pharmacol 122:208–213.

Wang J, Lewis DM, Castranova V, Frazer DG, Goldsmith T, Tomblyn S, et al. 2001. Characterization of asphalt fume composition under simulated road paving conditions by GC/MS and microflow LC/quadrapole time-of-flight MS. Anal Chem 73:3961–3970.

Watts RR, wallingford KM, Williams RW, House DE, Levtas J. 1998. Airborne exposures to PAH and PM10 particles for road paving workers applying conventional asphalt and rubber modified asphalt. J Expos Anal Environ Epidemiol 8:213–229.

Wiley JC, Cey EL, Frampton MW, Torres A, Apostolakos MJ, Hoehn G, et al. 1997. Quantitative RT-PCR measurement of cytochromes p450 1A1, 1B1, and 2B1, microsomal epoxide hydrase, and NADPH oxidoreductase expression in lung cells of smokers and non-smokers. Am J Respir Cell Mol Biol 17:114–124.

Yang HM, Barger MW, Castranova V, Ma JY, Yang JJ, Ma JY. 1999. Effects of diesel exhaust particles (DEP), carbon black, and silica on macrophage responses to lipopolysaccharide: evidence of DEP suppression of macrophage activity. J Toxicol Environ Health 58:261–278.

Yang HM, Ma JY, Castranova V, Ma JY. 1997. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor-alpha from rat alveolar macrophages. Exp Lung Res 23:289–294.