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ALTERATION OF PULMONARY CYTOCHROME P-450 SYSTEM: EFFECTS OF ASPHALT FUME CONDENSATE EXPOSURE

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Exposure to asphalt fumes is a health concern due to the presence of polycyclic aromatic compounds (PACs) in asphalt. Bioactivation of many PACs requires metabolism by the cytochrome P-450 (P-450) system. The objective of this study was to evaluate the effects of exposure of rats to asphalt fume condensate (AFC), collected at the top of a paving asphalt storage tank, on the pulmonary microsomal P-450 system and to determine the genotoxic effects of such exposure. Male Sprague-Dawley rats were intratracheally instilled with saline or with 0.45, 2.22, or 8.88 mg/kg AFC for 3 consecutive days and sacrificed the following day. Lung microsomes were isolated by differential centrifugation of lung homogenates. Microsomal protein level, NADPH cytochrome c reductase activity, and the activities and protein levels of cytochrome P-450 isozymes CYP1A1 and CYP2B1 were monitored to assess the effects of AFC exposure on pulmonary P-450. The activities of CYP2B1 and CYP1A1 were determined by monitoring xenobiotic metabolism of 7-pentoxyresorufin and 7-ethoxyresorufin, respectively. CYP2B1 and CYP1A1 levels were determined by immunochemical analysis. Micronucleus (MN) formation in bone-marrow polychromatic erythrocytes (PCEs) was determined to assess the genotoxic effects of AFC exposure. The results showed that exposure of rats to AFC did not significantly affect total cytochrome P-450 content or cytochrome c reductase activity in the lung. CYP2B1 levels and enzyme activity were not significantly affected by AFC exposure. In contrast, CYP1A1 levels and activity were significantly increased in microsomes isolated from AFC-exposed lungs. Increased MN formation was observed only in high-dose AFC-exposed bone marrow PCEs. These results demonstrate that AFC exposure induced CYP1A1 activity and increased the enzyme levels of CYP1A1 in lung microsomes, suggesting that AFC exposure may alter metabolism of PACs by the cytochrome P-450 system in the lung. Alteration of cytochrome P-450 metabolism of PACs may contribute to the AFC-induced genotoxic effects demonstrated as MN formation.

Asphalt is composed of a complex mixture of aliphatic paraffinic and aromatic hydrocarbons including heteroatomic compounds containing sulfur, nitrogen, and oxygen. Asphalt composition varies depending on the source of the crude oil and the end use. In the United States, asphalt is mainly used for road paving and much of the remainder is used in the man-

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ufacture and installation of roofing products (Asphalt Institute, 1990). Approximately 300,000 workers are employed in the asphalt paving industry in the United States (Burr & Miller, 1996). Inhalation and dermal exposure are believed to be the primary routes of exposure for asphalt pavers.

Exposure of animals to paving-temperature asphalt fume condensate did not induce significant lung injury (Ma et al., 2000). A recent epidemological study reported that asphalt fume exposure did not produce significant changes in lung function and symptoms among road pavers (Gamble et al., 1999). However, the potential carcinogenic effects of asphalt fumes have not been fully addressed.

Due to the presence of polycyclic aromatic compounds (PACs), asphalt fumes are potentially carcinogenic and may pose health risks to the exposed workers (International Agency for Research on Cancer, 1985). Epidemiological studies have shown that there is a consistently higher risk of cancer development for roofers than road pavers (Partanen & Boffetta, 1994). This is consistent with the fact that the asphalt fumes generated at lower paving temperatures (~160°C) contains much less PACs than asphalt fumes generated at higher roofing temperatures (~232°C) (Machado et al., 1993). Machado et al. (1993) have also shown that there is a good correlation between mutagenicity and the three- to seven-ring PAC content in asphalt fumes using a modified Ames test.

Even though the exposure to PACs through asphalt fumes is low for the road workers (Monarca et al., 1987; Watts et al., 1998), enhanced levels of urinary excretion of 1-hydroxypyrene from road-paving asphalt workers have been determined (Burgaz et al., 1992; Jarvholm et al., 1999), suggesting that there may be an increased genotoxic risk for these workers.

Pulmonary cytochrome P-450 enzymes play a major role in the metabolism of xenobiotics and are responsible for detoxification of many xenobiotics. However, some PACs require bioactivation to form reactive metabolites and to exert their toxic and/or mutagenic activities. In the rat lung, the isozyme CYP2B1 and NADPH cytochrome P-450 reductase are the major constituents of the pulmonary cytochrome P-450 system under basal conditions (Christou et al., 1987). In contrast, the isozyme CYP1A1 is present at a very low concentration in the unexposed lung. However, CYP1A1 is highly inducible by PACs. Induced CYP1A1 can then metabolize PACs to ultimate carcinogenic bay-region diol epoxide metabolites (Shimada et al., 1992; Whitlock, 1999). The presence of PACs in asphalt fumes suggests that this fume exposure may alter cytochrome P-450 systems and PAC metabolism in the lung.

The induction of micronuclei is an indirect marker for structural or numerical chromosomal change (Heddle et al., 1991), which may be used as an indicator for the carcinogenic potential of chemicals or complex mixtures such as asphalt fumes. Studies have shown that exposure of road workers to paving asphalt fumes did not induce significant DNA strand breaks in peripheral mononuclear blood cells (Fuchs et al., 1996), or signifi-

cant sister chromatid exchanges or micronuclei formation in peripheral lymphocytes (Jarvholm et al., 1999).

At the present time, there is little information concerning the effects of exposure to paving asphalt fume on pulmonary xenobiotic metabolism. This study was carried out to determine the effects of intratracheal instillation of asphalt fume condensate, collected at the top of paving asphalt storage tank, on the pulmonary microsomal cytochrome P-450 monooxygenase system and to monitor possible genotoxic effects of such exposure in a rat model. The specific objectives were to assess the effects of exposure of rats to paving asphalt fume condensate on: (1) the two major components of the cytochrome P-450 system, namely, cytochrome P-450 and NADPH cytochrome P-450 reductase; (2) the levels of two major isozymes of cytochrome P-450, CYP1A1 and CYP2B1; (3) the metabolic activities of these isozymes; and (4) the formation of micronuclei in bone-marrow polychromatic erythrocytes.

METHODS

Asphalt Fume Condensate

Asphalt fume condensate (AFC) was collected at the top of a paving storage tank at Asphalt Materials, Indianapolis, IN. The paving asphalt, a PG 64-22 used on the I-65 (1997) project, was collected at 160°C. Asphalt fume condensate was collected by cold trap using the same pumps and traps as used in the laboratory fume generator described by Sivak et al. (1997). The composition of the AFC from this storage tank was characterized by fluorescence analysis, gas chromatography with flame ionization detection (GC/FID) for simulated distillation, and gas chromatography/mass spectrometry (GC/MS) and was found to be similar to the composition of condensate collected from road paving operations (Kriech et al., 1999).

Treatment of Animals

Male Sprague-Dawley rats, Hla:(SD)CVF, from Hilltop Lab Animals (Scottdale, PA), monitored free of endogenous viral pathogens, parasites, mucoplasmas, *Helicobacter* and CAR *Bacillus,* weighing about 150 g (approximately 5 wk old) at arrival, were used for all experiments. The rats were acclimated for 1 wk before use and were kept in filtered ventilated cages on Alpha-dri virgin cellulose chips and hardwood Beta-chips as bedding, provided with HEPA-filtered air, autoclaved Prolab 3500 diet, and tap water ad libitum, under controlled light cycle (12 h light/12 h darkness) and temperature (22–24°C) conditions. Facilities were AAALAC accredited, specific pathogen free, and environmentally controlled. Animals were anesthetized with sodium methohexital (brevitol; Eli Lilly Co., Indianapolis, IN) and intratracheally (IT) instilled with saline or AFC. Rats received an IT instillation of 0.45, 2.22, or 8.88 mg AFC/kg body weight (i.e., approximately 0.1, 0.5, or 2 mg AFC/0.25 ml sterile saline/rat) or vehicle alone as control for 3 consecutive days and were sacrificed the following day. These doses of asphalt fume

condensate have been shown previously to cause no significant changes in pulmonary inflammation, lung damage, or alveolar macrophage activity (Ma et al., 2000).

Isolation of Microsomes

The rats were anesthetized with pentobarbital sodium (150 mg/kg body weight), and the heart and lungs were removed. The lungs were perfused with saline solution to remove blood cells. The lung tissue was chopped 4 times with a McIlwain tissue chopper (Mickle Engineering Co., Gomshall, Surrey, UK) set at 0.5 mm slice thickness. The minced lungs were suspended in 4 times lung weight of ice-cold incubation medium (145 m*M* KCl, 30 m*M* Tris-HCl, 1.9 m*M* KH_2PO_4, 8.1 m*M* K_2HPO_4, and 3 m*M* $MgCl_2$, pH 7.4) and homogenized using a Teflon–glass Potter–Elvejhem homogenizer for 16 complete passes. The microsomal fraction of the tissue homogenate was obtained by differential centrifugation as described previously (Miles et al., 1996). The microsomal pellet was resuspended in incubation medium at a tissue concentration of 1 g/ml. The protein concentration of the microsomal fraction was determined by using a BCA protein assay kit (Pierce, Rockford, IL).

Cyochrome P-450 Content and NADPH Cyochrome *c* Reductase Activity

Total cytochrome P-450 content and NADPH cytochrome *c* reductase activity were measured in lung microsomes obtained from control and AFC-treated rats. Total P-450 content was measured by the dithionite difference technique spectrophotometrically between 450 and 500 nm (Matsubara et al., 1974). Before the measurement, argon gas was gently bubbled through the suspensions for 1 min to minimize the spectral interference of oxy- and deoxyhemoglobin. The extinction coefficient used was 100 mM^{-1} cm^{-1} (Estabrook et al., 1972). The results were expressed as picomoles/milligram microsomal protein.

NADPH cytochrome *c* reductase activity was determined by monitoring the rate of reduction of cytochrome *c* at 550 nm (Masters et al., 1967) in lung microsomes using a Gilford Response II spectrophotometer (CIBA-Corning Diagnostics Corp., Oberlin, OH). To prevent any oxidation of reduced cytochrome *c* by potential mitochondrial contamination, 2.2 m*M* KCN was added to the microsomal suspensions. The reaction was initiated by the addition of 50 μ*M* NADPH. An extinction coefficient of 18.7 mM^{-1} cm^{-1} was used (Masters et al., 1967), and the results were expressed as nanomoles cytochrome *c* reduced/milligram microsomal protein/min.

CYP1A1 and CYP2B1 Activities

7-Pentoxyresorufin and 7-ethoxyresorufin are known to be metabolized by CYP2B1 and CYP1A1, respectively. CYP2B1 and CYP1A1 activity were monitored by measuring the *O*-dealkylation of 7-pentoxyresorufin (PROD)

and 7-ethoxyresorufin (EROD) in microsomal suspensions containing 0.1 mg microsomal protein/ml incubation medium (pH 7.6), 5 μ*M* 7-PR or 3 μ*M* 7-ER (Sigma Chemical Co., St. Louis, MO) in a total of 3 ml, and the reaction was initiated by addition of 0.48 m*M* NADPH at 37°C. The activities of PROD and EROD were measured by monitoring the formation of resorufin spectrafluorometrically at an excitation wavelength of 530 nm and emission of 585 nm (model LS-50 luminescence spectrometer, Perkin-Elmer Corp., Norwalk, CT) according to the method of Burke and Mayer (1974). The results were expressed as picomoles resorufin formed per minute per milligram microsomal protein.

Immunochemical Analysis

Lung microsomal proteins, at equivalent amounts (20 μg for CYP2B1 and 100 μg for CYP1A1) of each sample, were subjected to discontinuous polyacrylamide gel electrophoresis in the presence of 10% sodium dodecyl sulfate (PAGE-SDS) for separation. Following electrophoresis, the proteins were transferred by electroblotting from the gel to nitrocellulose membrane, according to the manufacturer's instruction (Novex, San Diego, CA). CYP1A1 and CYP2B1 were detected by immunochemical reaction using commercial kits purchased from Amersham (Piscataway, NJ) following the manufacturer's instruction. The proteins bound to the nitrocellulose membrane reacted with a rabbit polyclonal antibody, prepared against rat microsomal CYP1A1 or CP2B1 at 4°C overnight. The membrane was then incubated with an anti-rabbit immunoglobulin (Ig)-biotinylated secondary antibody, followed by an incubation with a streptavidin–horseradish peroxidase conjugate, which binds to the biotinylated secondary antibody. The immunocomplexes were visualized by the enhanced chemiluminescence method. The intensity of the protein bands on the x-ray films was scanned using the Stratagene Eagle Eye II (La Jolla, California) with Eagle Sight software. After scanning, the density was measured using ImageQuant 5.1 software from Molecular Dynamics (Amersham Pharmacia Biotech, Piscataway, NJ).

Micronucleus Assay

The nucleated cells in bone marrow were isolated, using a cellulose column (Romagna & Staniforth, 1989). The erythrocytes collected from cellulose column were pelleted by centrifugation (600 × g, 10 min) and resuspended in fetal bovine serum. An aliquot of cell suspension was pelleted onto slides using a Shandon Cytospin II at 600 rpm for 7 min. The slides were allowed to air-dry, were fixed in absolute methanol, and were stained with Giemsa–distilled water (1:6) for 10 min. All slides were scored under 1000× magnification. The polychromatic erythrocytes (PCEs) showed a strong bluish tint. For each animal, the frequency of micronucleated polychromatic erythrocytes (MNPCEs) in bone marrow was based on 10,000 PCEs scored. The results were expressed as the mean number of cells with micronuclei per 1000 PCEs.

Statistical Analysis

Results were expressed as means ± standard error (SE) from at least five different animals. Statistical analyses were carried out with the JMP IN statistical program (SAS, Inc., Belmont, CA). The significance of the interaction among the different treatment groups was assessed using analysis of variance (ANOVA) and analyzed using the Tukey–Kramer post hoc test. Significance was set at $p < .05$. The comparisons of MNPCEs between control and AFC-treated groups were carried out by the trend test at $p < .05$. The significance between different dose groups of treated animals and the control was evaluated by chi-square analysis. The group *t*-test for multiple samples was used to compare PCEs between treated and control animals.

RESULTS

Lung Weight and Microsomal Protein Content

Lung weights and total microsomal protein contents of the lungs from saline control and AFC-exposed rats were monitored to assess the gross effects of AFC exposure on the lung (Table 1). The results show that intratracheal exposure of rats to AFC for 3 consecutive days at 3 different concentrations (0.45, 2.22, or 8.88 mg/kg) did not produce significant effects on lung weight or the amount of total microsomal protein in the lung. The body weight of rats was not affected by the AFC exposure (data not shown).

Microsomal Cytochrome P-450 Content and NADPH Cytochrome *c* Reductase Activity

The effects of exposure of rats to AFC on total cytochrome P-450 content and the activity of NADPH cytochrome *c* reductase were monitored as shown in Table 2. The results show that exposure of rats to AFC at 0.45, 2.22, or 8.88 mg/kg for 3 consecutive days did not significantly affect total cytochrome P-450 content in lung microsomes. In addition, the activity of NADPH cytochrome *c* reductase was not significantly altered by the asphalt fume condensate exposure.

TABLE 1. Lung Weight and Microsomal Protein Content in Lungs from Control and Asphalt Fume Condensate (AFC)-Treated Rats

Exposure	Lung weight (g)	Microsomal protein (mg/g lung)	Total microsomal protein (mg/lung)
Saline	1.23 ± 0.07	5.80 ± 0.31	6.29 ± 0.92
AFC, 0.45 mg/kg; 3 times	1.17 ± 0.05	4.91 ± 0.18	5.76 ± 0.29
AFC, 2.22 mg/kg; 3 times	1.43 ± 0.08	4.65 ± 0.33	6.62 ± 0.59
AFC, 8.88 mg/kg; 3 times	1.20 ± 0.03	6.05 ± 0.26	7.28 ± 0.36

Note. Values are mean ± SE for data from at least five different animals in each treatment group. Rats were exposed to saline or 0.45, 2.22, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day.

TABLE 2. Effects of Exposure of Rats to Asphalt Fume Condensate (AFC) on Cytochrome P-450 Content, Total Cytochrome P-450 Content, and NADPH Cytochrome *c* Reductase Activity in Lung Microsomes

Treatment	P-450 (pmol/mg protein)	Total P-450 (pmol/lung)	NADPH cytochrome *c* reductase (nmol/min/mg protein)
Saline	112 ± 24	574 ± 88	9.68 ± 1.95
AFC, 0.45 mg/kg; 3 times	79 ± 5	475 ± 30	8.38 ± 0.78
AFC, 2.22 mg/kg; 3 times	83 ± 13	440 ± 46	8.54 ± 1.49
AFC, 8.88 mg/kg; 3 times	102 ± 17	698 ± 21	9.05 ± 0.47

Note. Values are mean ± SE for data from at least five different animals in each treatment group. Rats were exposed to saline or 0.45, 2.22, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day.

Activities of CYP1A1 and CYP2B1

The activities of 7-pentoxyresorufin *O*-dealkylase (PROD) and 7-ethoxyresorufin *O*-dealkylase (EROD) were measured in the pulmonary microsomal fraction from control and AFC-exposed rats. Figures 1 and 2 show the metabolism of 7-PR and 7-ER, respectively, by lung microsomes. The *O*-dealkylase activity was determined by monitoring the production of resorufin. The results show that exposure of rats to AFC did not significantly affect the 7-PR metabolism by lung microsomes. Figure 2 shows that the metabolism of 7-ER was significantly increased in microsomes isolated from AFC-exposed rats.

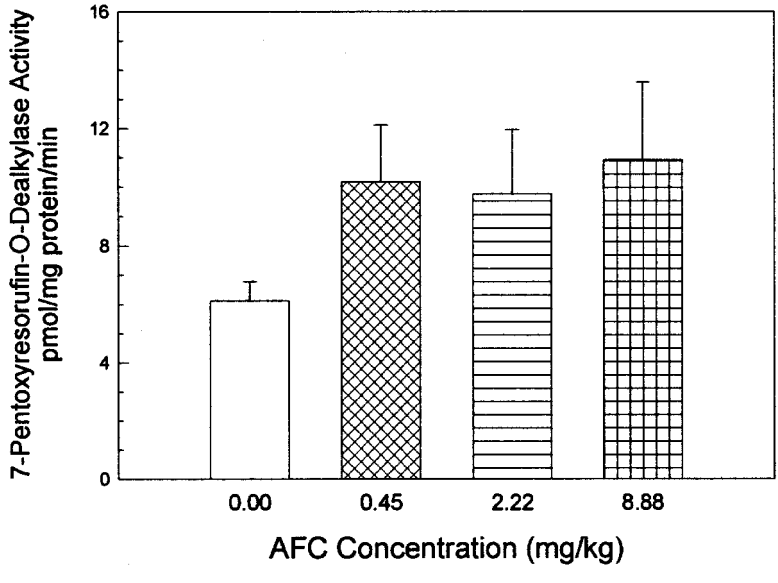

FIGURE 1. Effects of AFC exposure on 7-pentoxyresorufin *O*-dealkylase activity. Rats were exposed to saline or to 0.45, 2.22, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day. CYP2B1 activity was monitored by measuring resorufin production by *O*-dealkylation of 7-pentoxyresorufin in microsomal suspensions. Values represent means ± SE ($n = 5$).

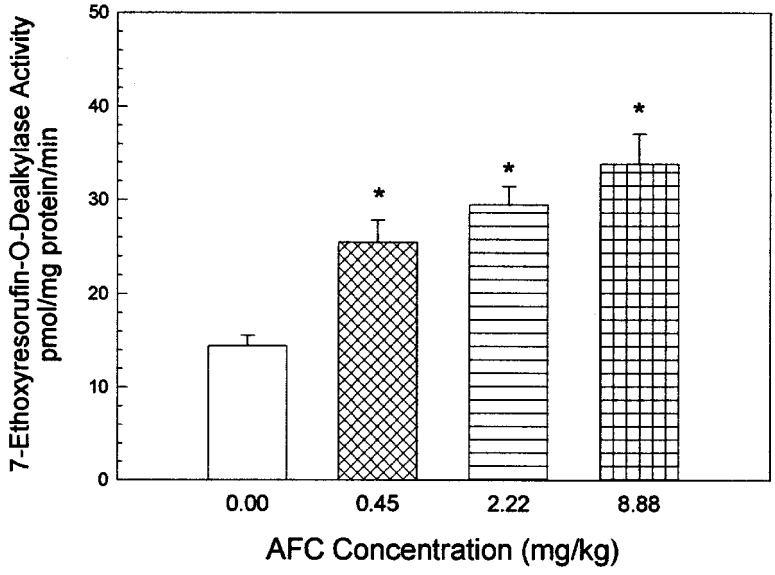

FIGURE 2. Effects of AFC exposure on 7-ethyoxyresorufin *O*-dealkylase activity. Rats were exposed to saline or 0.45, 2.22, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day. CYP1A1 activity was monitored by measuring resorufin production by *O*-dealkylation of 7-ethyoxyresorufin in microsomal suspensions. Values represent means ± SE (n = 5–9). Asterisk indicates a significant difference from the saline control group, $p < .05$.

Cytochrome P-450 Isozyme CYP1A1 and CYP2B1 Protein Levels

The protein levels of CYP1A1 and CYP2B1 in microsomes from control and AFC-exposed rats were determined by Western blot analysis as shown in Figure 3. The results show that AFC exposure did not induce a significant change in the CYP2B1 protein level in comparison to control. In contrast, the exposure of rats to AFC (8.88 mg/kg for 3 consecutive days) significantly increased the microsomal CYP1A1 protein level in the lung.

Induction of Micronuclei

Table 3 shows that a similar number of polychromatic erythrocytes (PCEs) were isolated from the bone marrow of rats exposed to a low concentration of AFC (0.45 mg/kg for 3 consecutive days) in comparison to the control. However, there was a significantly lower number of PCEs in the high-dose AFC (8.88 mg/kg for 3 consecutive days)-exposed rats than in the control. The results also show that there was a significant increase in the frequency of micronuclei formation in the high-dose AFC-exposed bone-marrow PCEs compare to the control, whereas the low-dose AFC exposure did not significantly affect the number of micronuclei in PCEs.

DISCUSSION

The effects of asphalt fumes on the pulmonary system have not been fully characterized. Studies have shown that asphalt fume induced subjec-

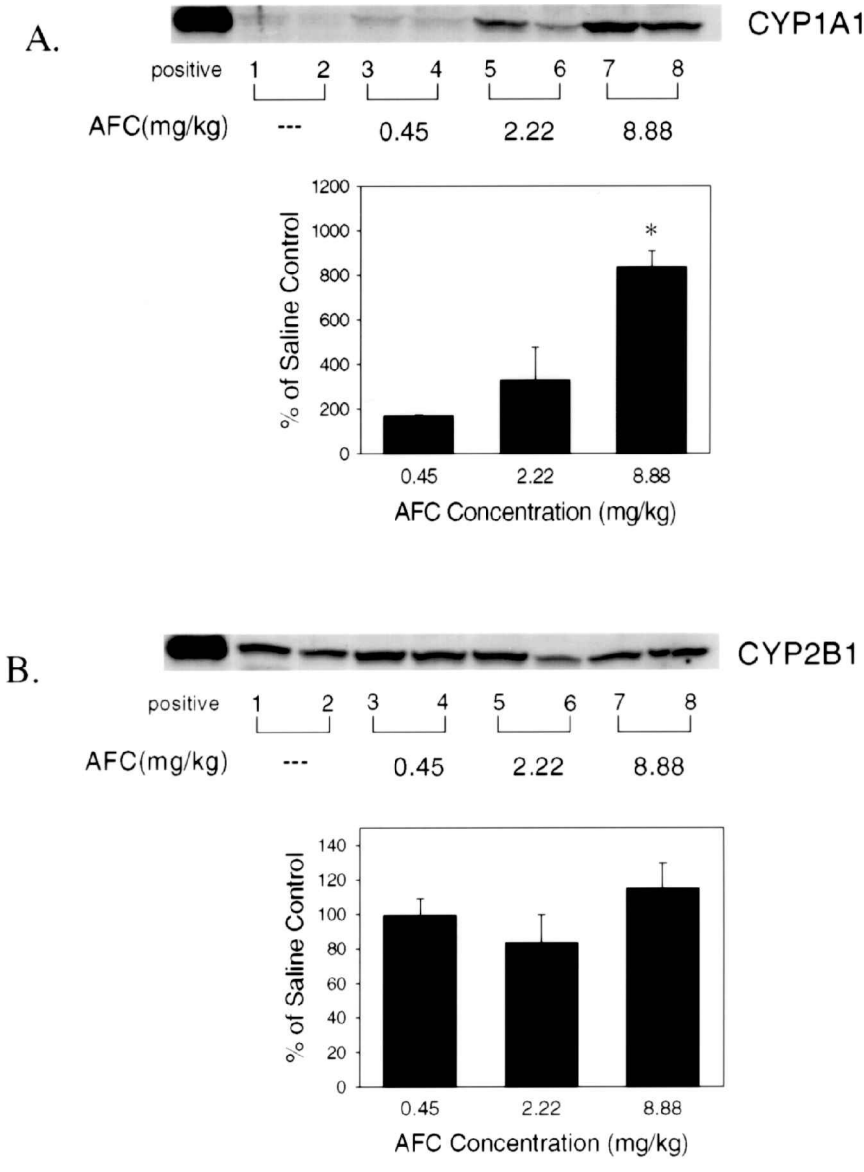

FIGURE 3. Western blot of lung microsomal fractions with rabbit polyclonal anti-rat CYP1A1 or CYP2B1 antibody. Rats were exposed to saline or 0.45, 2.22, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day. Microsomal protein was isolated from saline control and AFC-exposed rats. Either 100 µg or 20 µg lung microsomal protein was loaded for (A) CYP1A1 or (B) CYP2B1 separation, respectively. Lung microsomal proteins from positive control (lane positive), saline control (lane 1, 2), 0.45 mg/kg AFC-treated (lane 3, 4), 2.22 mg/kg AFC-treated (lane 5, 6), and 8.88 mg/kg AFC-treated (lane 7, 8) rats were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and detected by a chemiluminescence method. Positive controls used were provided by the supplier and were used according to the manufacturer's instructions. The protein signals of CYP1A1 and CYP2B1 were quantified by densitometric analysis and presented as a percentage of the saline control. Results are representative of at least three independent experiments. Asterisk indicates a significant difference from the saline control group, $p < .05$.

TABLE 3. Induction of Micronuclei (MN) in Bone-Marrow Polychromatic Erythrocytes (PCEs) Following Asphalt Fume Condensate (AFC) Exposure in Comparison to the Control

Treatment[a]	Number of MN/1000 PCEs[a]	Number of PCEs in 1000 erythrocytes	PCE/NCE ratio
Saline	1.5 ± 0.4	499 ± 13	1.0 ± 0.05
AFC, 0.45 mg/kg; 3 times	2.0 ± 0.4	468 ± 22	0.90 ± 0.07
AFC, 8.88 mg/kg; 3 times	2.9 ± 0.6[b]	440 ± 8[c]	0.79 ± 0.03

Note. Values are mean ± SE for data from five different animals in each treatment group. Rats were exposed to saline, 0.45 mg/kg AFC, or 8.88 mg/kg of AFC by intratracheal instillation for 3 consecutive days and sacrificed the next day. NCE, normal chromatic erythrocytes.

[a]For each treatment group, 10,000 PCEs were scored.

[b]Significantly different from controls by chi-square test at $p < .05$ and trend test valued $z = 2.145$, $p < .05$.

[c]Significantly different from controls by group *t*-test for multiple samples, $p << .05$.

tive symptoms (Norseth et al., 1991) and nonmalignant pulmonary effects, such as bronchitis, emphysema, and asthma, in some paving workers (Maizlish et al., 1988). However, in a recent study, Gamble et al. (1999) reported that there is no consistent association between the level of exposure to asphalt fumes and the acute reduction in lung function or the incidence of symptoms among road workers. Previous studies carrier out in this laboratory showed that exposure of rats to asphalt fume condensate, collected from a paving asphalt storage tank, did not cause significant pulmonary inflammation or lung injury (Ma et al., 2000), which is probably due to the fact that AFC does not contain particles that are known to activate macrophage respiratory burst activity.

The potential carcinogenic and/or mutagenic effects of AFC have been the subject of many studies, but the effects of AFC on the cytochrome P-450 monooxygenase system in the lung have not been clearly demonstrated. This study was carried out to characterize the effects of AFC, which has a composition similar to condensate collected at road paving sites (Kriech et al., 1999), on the pulmonary cytochrome P-450, at concentrations that did not induce pulmonary inflammation or damage. The results show that exposure of rats to AFC did not significantly affect the total cytochrome P-450 content in the lung or the NADPH cytochrome *c* reductase activity. However, AFC clearly had an effect on the pulmonary microsomal P-450 systems. The protein level and enzyme activity of CYP2B1, a constitutive enzyme believed to be responsible for the detoxification processes, were not significantly affected by AFC exposure. In contrast, AFC exposure significantly increased CYP1A1 enzyme levels and enhanced CYP1A1 activity of lung microsomes. It is known that CYP1A1 can be induced by its substrate, PACs, and is responsible for the transformation of PACs to mutagenic species and to proximate and ultimate carcinogenic metabolites (Whitlock, 1999). Studies of PAC metabolism, mutagenicity, and DNA binding show that benzo-ring diol epoxides at the bay region of PACs are the principal ulti-

mate carcinogens (Shimada et al., 1992; Whitlock, 1999). Induction of aryl hydrocarbon hydroxylase (AHH) activity by certain drugs and xenobiotics in rat lung, such as by β-naphthoflavone, has been demonstrated (Jones et al., 1982). These results suggest that AHH activity is associated with CYP1A1. Most phenolic metabolites of PACs are nonmutagenic, but some of these metabolites are premutagens that may undergo metabolic activation by cytochrome P-450 to form mutagenic products. Thus, induction of CYP1A1 may lead to more mutagenic metabolites.

For conventional asphalt pavers, the exposure to PACs was very low when assessed using a personal exposure monitoring system (Monarca et al., 1987) or a biological monitoring system (Burgaz et al., 1992; Jarvholm et al., 1999) using benzo[a]pyrene (BaP) as a carcinogen marker. However, these workers were also exposed to elevated airborne concentrations of a group of unidentified compounds that may contain potentially carcinogenic PACs, such as benz[a]anthracence, chrysene, and the methylated derivatives of both (Watts et al., 1998). Studies have shown that the carcinogenic effect of BaP on mouse skin can be enhanced by concomitant administration of benzo[e]pyrene, fluoranthene, pyrene, and other PACs (Hermann, 1981). Thus, even though the amount of the procarcinogenic PAC, such as BaP, in paving fume is low and seems unlikely by itself to induce carcinogenic/mutagenic effects, the synergistic effects of other PACs on mutagenic activity through CYP1A1 induction could significantly enhance the carcinogenic/mutangenic responses to asphalt fume exposure. Indeed, several studies have demonstrated that the carcinogenic potency associated with environmental exposures to PACs cannot be simply determined by their BaP content (Garshick et al., 1987; Pott & Heinrich, 1990). In both cigarette smoking (Bartsch et al., 1999; Kiyohara et al., 1998) and diesel exhaust exposure (Garshick et al., 1987; Pott & Heinrich, 1990), the incidence of tumor formation or lung cancer is linked to the PAC exposure but is not directly related to the BaP content.

A number of studies have shown that roofers exhibit a greater risk for the lung and stomach cancers than road paving workers (Partanen & Boffetta, 1994). In addition, substantial genotoxic damage in peripheral mononuclear blood cells was detected in exposed roofers but not in road pavers (Fuchs et al., 1996). These results have been attributed to the fact that roofing asphalt fume contains higher levels of PACs than the paving asphalt fumes (Machado et al., 1993). Animal studies have shown that laboratory-generated roofing asphalt fume condensate exposure induced a higher frequency of micronucleated cells in cultured mammalian cells (Qian et al., 1996, 1999) and elevated DNA adduct formation in lung cells (Qian et al., 1998). However, this laboratory-generated roofing asphalt fume condensate may contain 5 to 100 times higher levels of three- and four-ring polycyclic aromatic sulfur heterocyclic compounds than condensate collected from storage tanks (Reinke et al., 2000). Thus, more studies are warranted to delineate the potential mutagenic activities of both roofing and paving asphalt fumes.

The present study shows that exposure of rats to a condensate of asphalt fume generated at paving temperatures resulted in a significant increase in micronucleated cell formation in bone marrow polychromatic erythrocytes. This paving AFC-induced mutagenic effect is comparable to the frequencies of micronuclei determined in rat bone marrow and spleen polychromatic erythrocytes of rats treated with dibenz[a,i]pyrene (Zhong et al., 1995), suggesting that exposure to paving asphalt fume may indeed increase the genotoxic risk for the exposed workers.

The exposure regime employed in this investigation, intratracheal instillation of relatively high doses of asphalt fume condensate, would result in a much higher delivery of asphalt-associated compounds to the lung than would be found under workplace conditions. However, exposure in this study was short (3 days) and responses acute (1 day postexposure). It is therefore difficult to extrapolate to the low-dose, chronic exposure of pavers to asphalt fumes. For this reason, the data presented in this study cannot be used for risk assessment, but are rather the first step in hazard identification. An inhalation study is currently underway to assess the effects of asphalt fume generated under paving conditions on the cytochrome P-450 system. This inhalation exposure resulted in much lower asphalt burdens to the lung, yet produced qualitatively similar induction of CYP1A1 (Ma et al., 2001). Further investigation of the time course and dose dependence of this induction is been conducted.

In summary, exposure of rats to AFC collected at paving temperature did not significantly affect lung weight, microsomal tissue concentration, P-450 content, or NADPH cytochrome *c* reductase activity, but significantly enhanced both the CYP1A1 level and CYP1A1 activity in the lung. However, the constitutive isozyme CYP2B1 content and activity in the lung were not affected by AFC exposure. In addition, exposure to a high concentration of AFC also induces micronuclei formation in bone-marrow polychromatic erythrocytes.

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