Journal of Toxicology and Environmental Health, Part A Current Issues

ISSN: 0098-4108 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh19

Chronic inhalation of diesel exhaust and coal dust: Effect of age and exposure on selected enzyme activities associated with microsomal cytochrome p-450 in rat lung and liver

Jean Rabovsky , Martin R. Petersen , Trent R. Lewis , Karl J. Marion & Robert D. Groseclose

CHRONIC INHALATION OF DIESEL EXHAUST AND COAL DUST: EFFECT OF AGE AND EXPOSURE ON SELECTED ENZYME ACTIVITIES ASSOCIATED WITH MICROSOMAL CYTOCHROME P-450 IN RAT LUNG AND LIVER

Jean Rabovsky, Martin R. Petersen, Trent R. Lewis, Karl J. Marion, Robert D. Groseclose

National Institute for Occupational Safety and Health, Morgantown, West Virginia

Male Fisher-344 rats were exposed by inhaltion to low levels of diesel exhaust and coal dust, alone or in combination, or to filtered air, 7 h/d, 5 d/wk for 24 mo. Cytochrome P-450-associated benzo[a]pyrene hydroxylase and 7-ethoxycoumarin deethylase activities were assayed in lung and liver microsomes after 3, 6, and 24 mo. Age-related changes in enzyme activities were observed, but they were not altered by the exposures. When the data were adjusted for age, only one difference was observed. Lung benzo-[a]pyrene hydroxylase activity in rats exposed to diesel exhaust and coal dust in combination was lower than that in animals exposed to coal dust alone (2.8 versus 4.4 pmol/min·mg protein). Neither value, however, differed significantly from the filtered-air controls, and no differences were observed in the other lung and liver activities. The data suggest exposure of the rats to diesel exhaust and/or coal dust had little or no effect on the selected lung and liver cytochrome P-450 activities under the conditions of the experiment.

INTRODUCTION

The use of diesel-powered equipment has raised concern over potential health effects on exposed populations, and studies have been carried out to evaluate the effect of diesel exhaust (DE) on physiological (Karagianes et al., 1981; Pepelko and Peirano, 1983) and biochemical processes (Lee

A preliminary account of this work was given at the First International Symposium on Foreign Compound Metabolism, Oct. 31–Nov. 4, 1983, Palm Beach, Fla.

The authors thank the following for support: animal sacrifice and tissue processing, Marilyn Gamble, Gary Harris, William Pailes, Charles Turick, and Crain White (NIOSH), and Karen Brook, Janet Lucas, Robert Long, and Edward McCabe (Ebon Research Systems); typing, NIOSH/LIB secretarial staff.

Although the research described in this article has been funded wholly or in part by the U.S. Environmental Protection Agency (EPA) through Interagency Agreement EPA-78-R-X0330 to National Institute for Occupational Safety and Health (NIOSH), it has not been subjected to EPA review and therefore does not necessarily reflect the views of EPA, and no official endorsement should be inferred.

Requests for reprints should be sent to Jean Rabovsky, National Institute for Occupational Safety and Health, 944 Chestnut Ridge Road, Morgantown, West Virginia 26505.

Journal of Toxicology and Environmental Health, 14:655–666, 1984

et al., 1980; Navarro et al., 1981; Chen and Vostal, 1981). Biochemical studies often focus on the cytochrome P-450 (P-450) enzyme system because of its role in the metabolism of foreign substances (Gillette et al., 1972; Pelkonen and Vähäkangas, 1980; Ioannides and Parke, 1980) in tissues such as lung and liver (Matsubara et al., 1974; Testa and Jenner, 1976; Minchin and Boyd, 1983). In each tissue, the biological endpoint, detoxication or tissue damage due to bioactivation, will depend on the balance of the hydrophilic, less toxic products (detoxication) and the more reactive species that combine with tissue macromolecules (bioactivation) (Ioanides and Parke, 1980; Pelkonen and Vähängas, 1980). Changes in P-450 levels may therefore be indicative of alterations in a major metabolic pathway induced by exposure to foreign matter. Such alterations may result in either diminished or enhanced ability of exposed individuals to effectively detoxify foreign substances.

Our concern over health effects of diesel exhaust focused on a population engaged in a specific occupational activity: underground coal mining. We were concerned with the effect of current and projected use of diesel-powered equipment in underground coal mines (Green et al., 1983) and possible interactions between DE and coal dust (CD). Although biological activity of DE has been the focus of many studies, little data exist on its effects in combination with CD. In a recent inhalation study, chronic exposure to the combined pollutants resulted in lesions similar to those associated with simple coal workers' pneumoconiosis; malignant respiratory-tract tumors, however, were not found (Karagianes et al., 1981). Over a 2-yr period we investigated the effect of DE and CD alone or in combination on the microsomal P-450 system of rats exposed by inhalation. Our purpose was to evaluate the effect of chronic exposure to DE and CD on the P-450 complex under conditions that approximate on-site exposure and conform to federal regulations.

METHODS AND MATERIALS

Animals

Male Fischer-344 rats, 6 wk of age at the start of exposure and immunized against Sendai virus, were used. Temperature was maintained at 21–22°C; food and water were available except during times of exposure.

Exposures

Rats were exposed 7 h/d, 5 d/wk in inhalation chambers that were darkened to simulate their active nocturnal period.

The chambers were designed by Hinners et al. (1980). Four exposure regimens were used: filtered air (control) (FA); diesel exhaust (DE); coal dust (CD); and diesel exhaust plus coal dust (DECD). Total particulate levels were as follows: 2 mg/m^3 in DE exposures, 5 mg/m^3 in CD exposures, and

and 3 mg/m^3 in DECD exposures. *Respirable* particulates (<7 μm were maintained at 2 mg/m^3 in all exposures. Details of the chamber construction, generation of diesel emission and coal dust, and gas composition of the chambers have been described (Hinners et al., 1980) (Table 1).

Animal Sacrifice and Microsome Preparation

Rats were sacrificed by decapitation after exposure for 3, 6, and 24 mo. Microsomes were prepared from lung and liver by differential centrifugation according to procedures previously described (Danner-Rabovsky and Groseclose, 1982). The final washed microsomal pellets were suspended in 0.05 *M* Tris, pH 7.4, plus 0.25 *M* sucrose, and stored at −80°C until used. We determined that microsomal activities were stable at −80°C for long periods (Danner-Rabovsky and Groseclose, 1982) (Table 2).

TABLE 1. Atmospheric Concentrations of Major Chamber Components over the 24-Month Exposure Duration[a]

Component	Treatment			
	Filtered air	Coal dust	Coal dust plus[d] diesel exhaust	Diesel exhaust
Respirable particles (mg/m^3)	NM[b]	2.1 ± 0.4[c]	2.0 ± 0.3	1.9 ± 0.3
CO_2 (%)	0.07 ± 0.02	0.08 ± 0.04	0.17 ± 0.06	0.16 ± 0.04
CO (ppm)	2.0 ± 0.9	2.1 ± 0.8	10.3 ± 2.0	10.5 ± 2.3
NO (ppm)	0.08 ± 0.13	0.08 ± 0.28	7.6 ± 2.8	7.8 ± 3.1
NO_2 (ppm)	0.06 ± .04	0.07 ± 0.05	1.5 ± 0.5	1.5 ± 0.5
SO_2 (ppm)	NM	0.003 ± 0.05	0.5 ± 0.3	0.6 ± 0.4
NH_3 (ppm)	0.5 ± 0.6	0.6 ± 0.7	0.4 ± 0.3	0.6 ± 0.8

[a]Airborne concentrations in the filtered air and coal dust chambers arose from the same ambient filtered/conditioned air supply and thus should be almost identical, except for the coal dust that was introduced. Coal dust plus diesel exhaust and the diesel exhaust alone represent contributions from the same generation source, with the baghouse filtration in the former instance the only variable.

[b]Not monitored.

[c]The errors associated with the measurements are expressed as ± SD.

[d]In the coal dust plus diesel exhaust exposures, the animals received 1 mg/m^3 of respirable CD and diluted DE containing 1 mg/m^3 of diesel particulate, which was totally respirable and contained the same gaseous/vapor emission present in the diesel emission particulate only chambers. This atmosphere was achieved by passing half of a split stream of diluted emissions from a diesel engine through filters that totally removed the particulate but did not remove the gaseous/vapor components of the exhaust. Thus, when the two diluted diesel emission streams were recombined, the diesel particulate was half (1 mg/m^3) of that found in the diesel exhaust chambers and the gaseous/vapor components were essentially identical. Coal dust was introduced as 1 mg/m^3 or 2 mg/m^3 respirable dust (≤7 μm) diameter) in the coal dust plus diesel exhaust and coal dust exposure chambers, respectively, utilizing a Wright Dust Feeder. This table presents the 24-mo summary of major components monitored in the exposure chamber atmospheres; atmospheric concentrations were quite stable throughout the study, and these data accurately reflect airborne concentrations at other interim periods.

TABLE 2. Effect of Long-Term Storage at −80°C on Microsomal Cytochrome P-450 Activities of 24-Month Fischer-344 Rats[a]

Enzyme	Tissue	Specific activity	
		Fresh	8 Months at −80°C
BaPOH-ase[b]	Lung	5.0	5.4
	Liver	0.13	0.19
7ECdeEt-ase[c]	Lung	0.16	0.11
	Liver	0.21	0.21

[a]Rats used for this experiment were not members of the colony used in the exposure study. They were obtained separately from the Charles River Breeding Facility.

[b]Benzo[a]pyrene hydroxylase: lung, pmol/min•mg protein; liver, nmol/min•mg protein.

[c]7-Ethoxycoumarin deethylase: nmol/min•mg protein.

Assays

Benzo[a]pyrene hydroxylase (BaPOH-ase) was assayed by a modified fluorometric procedure (Nebert and Gelboin, 1968; Weibel et al., 1977), using 3-hydroxybenzo[a]pyrene as standard. 7-Ethoxycoumarin deethylase (7ECdeEt-ase) was assayed by the fluorometric procedure of Greenlee and Poland (1978) with 7-hydroxycoumarin as standard. Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard. Total P-450 was measured as the dithionite reduced carbon monoxide adduct (Estabrook et al., 1972). BaPOH-ase and 7ECdeEt-ase activities were measured at 37°C in 0.03 *M* HEPES, pH 7.6. The NADPH needed for the P-450 activities was supplied by a generating system that consisted of $NADP^+$, $MgCl_2$, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase (Bend et al., 1972; Danner-Rabovsky and Groseclose, 1982).

TABLE 3. Protocol for Analyzing Cytochrome P-450 Activities in Rats Exposed to Coal Dust and Diesel Exhaust

Exposures:	Filtered air (control), diesel exhaust, coal dust, diesel exhaust plus coal dust
Exposure interval:	3, 6, 24 Months
Sample size:	3 Groups/exposure at a given exposure interval, each group contained the pooled organs from 3 rats
Organs:	Liver, lung
Subcellular fraction:	Microsomes
Total content:	Microsomal protein; cytochrome P-450
Enzyme activities:	Benzo[a]pyrene hydroxylase, 7-ethoxycoumarin deethylase

Units of activity have been defined as nmol product/min for liver and lung 7ECdeEt-ase and liver BaPOH-ase and pmol/min for lung BaPOH-ase. Specific activity is defined as units/mg microsomal protein. Total protein has been expressed as mg microsomal protein/g tissue, and total P-450 as nmol P-450/mg microsomal protein.

The protocol for experimental procedures is described in Table 3.

Materials and Equipment

Chemicals were purchased from the following sources: Tris, HEPES, bovine serum albumin (crystallized and lyophilized), glucose 6-phosphate, glucose-6-phosphate dehydrogenase (Torula Yeast, Type XII) from Sigma Chemical Co. (St. Louis, Mo.); 7-ethoxycoumarin (gold label, 99.9+%), 7-hydroxycoumarin (95+%), benzo[a]pyrene, (99+% gold label) from Aldrich Chemical Co. (Milwaukee, Wis.). 3-Hydroxybenzo[a]pyrene was received from the NCI Chemical Carcinogen Reference Standard Repository, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Md. Other reagents were reagent grade. Deionized, glass-distilled water was used for all experiments.

Statistical Analysis

The data were treated as a completely randomized design with a 2 X 2 X 3 factorial arrangement of treatments, and each dependent variable was assumed to be normally distributed. The data wre analyzed by general linear hypothesis tests appropriate for analysis of variance (Searle, 1971), and Fisher's least significant differences test was used for comparing two means (Snedecor and Cochran, 1967). Data were analyzed by the Statistical Analysis System (SAS Institute, Inc., 1979), with the probability of a type I error set at $\alpha = 0.05$.

The following hypotheses were tested.

"The effect of exposure is the same for each time interval." (1)

If (1) was not rejected, we tested (2).

"The effect of CD (which is the same for each time interval is the same whether or not DE is present." (2)

If (2) was not rejected, we tested the following:

"The effect of CD (which is the same for all time intervals and the same in the presence or absence of DE) is zero (i.e., there is no effect)." (3)

"The effect of DE is zero." (4)

If hypothesis (1) was not rejected, we also tested:

"The effect of age is zero." (5)

If hypothesis (1) was concluded to be false, a separate 2 X 2 analysis should be carried out for each time period. If, however, hypothesis (1) was concluded to be true, the aforementioned method of analysis would have two advantages over the 2 X 2 analysis for each time interval. First, small differences may now be detectable. Second, less false positive results (type I errors) should be found. Similar considerations also pertain to hypothesis (2). Note also that, since the estimated effects of exposure are averages of age-specific effects, the estimates are adjusted for age. Similarly, the estimated age effects are adjusted for exposure.

RESULTS

The data obtained from P-450-associated microsomal activities are summarized in Tables 4-7. Tables 4 and 5 contain results from each type of exposure and each interval of exposure. Values shown are the means obtained from three samples, in which each sample contained the lungs or livers from three animals. Some of the differences appear to be statistically significant. However, for reasons stated in the methods section, exposure was not tested at each age interval and age was not tested at each exposure, unless the age-exposure interaction was significant.

When analysis of variance was performed, the age-exposure interactions

TABLE 4. Effect of Age and Exposure on Lung Microsomal Metabolism

Length of exposure[a] (mo)	Exposure	Protein[b]	BaPOH-ase[c]	7ECdeEt-ase[d]
3	FA	4.0 ± 0.60	2.4 ± 0.53	0.11 ± 0.040
	DE	4.6 ± 0.67	2.6 ± 0.57	0.061 ± 0.012
	CD	4.9 ± 0.85	2.6 ± 0.27	0.062 ± 0.028
	DECD	4.0 ± 0.30	2.6 ± 0.71	0.048 ± 0.018
6	FA	2.3 ± 0.20	3.3 ± 0.55	0.11 ± 0.012
	DE	2.7 ± 0.31	3.3 ± 0.73	0.11 ± 0.014
	CD	2.7 ± 0.33	5.3 ± 0.33	0.13 ± 0.017
	DECD	2.8 ± 0.26	3.4 ± 0.93	0.12 ± 0.024
24	FA	3.1 ± 0.11	4.4 ± 0.26	0.075 ± 0.0083
	DE	3.6 ± 1.2	4.6 ± 1.0	0.067 ± 0.016
	CD	3.7 ± 0.44	5.2 ± 1.1	0.091 ± 0.024
	DECD	3.2 ± 0.39	2.3 ± 0.43	0.039 ± 0.0028

[a]Rats were 6 wk of age at start of exposure.
[b]In mg/g tissue, ± SEM.
[c]Benzo[a]pyrene hydroxylase, pmol/min•mg protein, ± SEM.
[d]7-Ethoxycoumarin deethylase, nmol/min•mg protein, ± SEM.

TABLE 5. Effect of Age and Exposure on Liver Microsomal Metabolism

Lenght of exposure[a] (mo)	Exposure	Protein[b]	Cytochrome P-450[c]	BaPOH-ase[d]	7ECdeEt-ase[e]
3	FA	16 ± 0.23	0.74 ± 0.043	1.9 ± 0.097	0.72 ± 0.043
	DE	12 ± 3.3	0.69 ± 0.057	1.8 ± 0.68	0.53 ± 0.18
	CD	15 ± 1.1	0.78 ± 0.050	2.1 ± 0.41	0.75 ± 0.092
	DECD	15 ± 0.78	0.85 ± 0.046	3.0 ± 0.88	0.83 ± 0.16
6	FA	13 ± 1.3	0.69 ± 0.10	1.6 ± 0.18	0.79 ± 0.098
	DE	14 ± 1.1	0.72 ± 0.032	1.7 ± 0.15	0.99 ± 0.075
	CD	12 ± 0.85	0.67 ± 0.10	1.3 ± 0.33	0.88 ± 0.10
	DECD	12 ± 0.44	0.71 ± 0.068	1.2 ± 0.21	0.87 ± 0.039
24	FA	13 ± 0.98	0.48 ± 0.15	0.068 ± 0.015	0.32 ± 0.033
	DE	11 ± 1.5	0.52 ± 0.0050	0.056 ± 0.0041	0.24 ± 0.031
	CD	10 ± 0.47	0.41 ± 0.37	0.052 ± 0.0025	0.30 ± 0.018
	DECD	9.2 ± 0.39	0.47 ± 0.049	0.055 ± 0.0046	0.16 ± 0.044

[a]Rats were 6 wk of age at start of exposure.
[b]In mg/g tissue, ± SEM.
[c]In nmol/mg protein, ± SEM.
[d]Benzo[a]pyrene hydroxylase, nmol/min•mg protein, ± SEM.
[e]7-Ethoxycoumarin deethylase, nmol/min•mg protein, ± SEM.

TABLE 6. Effect of Age on Lung and Liver Microsomal Enzyme Activities[a]

Tissue	Exposure interval[b] (mo)	Cytochrome P-450[c]	BaPOH-ase[d]	7ECdeEt-ase[e]
Lung	3	[f]	2.6	0.069
	6		3.8	0.12
	24		4.1	0.068
	Pooled SD[g]		1.1	0.035
Lung comparison			3 < 6, 24	3, 24 < 6
Liver	3	0.76	2.2	0.71
	6	0.70	1.4	0.88
	24	0.47	0.058	0.26
	Pooled SD	0.10	0.65	0.16
Liver comparison		3, 6 > 24	3 > 6 > 24	6 > 3 > 24

[a]Values given for cytochrome P-450, BaPOH-ase, and 7ECdeEt-ase are means adjusted for exposure.
[b]Rats were 6 wk of age at start of exposure.
[c]In nmol/mg microsomal protein.
[d]Benzo[a]pyrene hydroxylase, pmol/min•mg protein, lung; nmol/min•mg protein, liver.
[e]7-Ethoxycoumarin deethylase, nmol/min•mg protein.
[f]Not done.
[g]Pooled standard deviation (SD) = (average of the estimated variances)$^{1/2}$.

TABLE 7. Effect ofExposure on Lung and Liver Microsomal Enzyme Activities[a]

Tissue	Exposure	BaPOH-ase[b]	7ECdeET-ase[c]
Lung	FA	3.4	0.098
	DE	3.5	0.078
	CD	4.4	0.095
	DECD	2.8	0.068
	Pooled SD[d]	1.1	0.035
Lung comparison		CD > DECD	NS[e]
Liver	FA	1.2	0.61
	DE	1.2	0.59
	CD	1.1	0.64
	DECD	1.4	0.62
	Pooled SD	0.65	0.16
Liver comparison		NS	NS

[a]Values given for BaPOH-ase and 7ECdeEt-ase are means adjusted for all intervals of exposure.
[b]Benzo[a] pyrene hydroxylase, pmol/min•mg protein, lung; nmol/min•mg protein, liver.
[c]7-Ethoxycoumarin deethylase, nmol/min•mg protein.
[d]Pooled standard deviation (SD) = (average of the estimated variances)$^{1/2}$.
[e]Not significant.

were not significant. Table 6 gives exposure-adjusted age means for enzyme activities, as well as a summary of conclusions from the adjusted means. Liver activities and total P-450 decreased over the 24-mo exposure period. Lung activities, on the other hand, did not show the same trend or consistency and appeared to increase after 3 mo.

When the exposure means were adjusted for age, an effect of exposure was observed for lung BaPOH-ase, such that less activity was measured in lung microsomes from animals exposed to DECD (specific activity = 2.8) than that measured in rats exposed to CD alone (specific activity = 4.4) (Table 7). In the absence of CD, no effect of DE was observed. The remaining parameters were not affected by the exposures.

DISCUSSION

Lung and liver P-450-associated microsomal activities were used to monitor the effects of inhaled DE and CD on detoxication/bioactivation processes. Lung was chosen because it is the initial entry point for inhaled pollutants. Liver is important because of its high P-450 content, which is sensitive to inhaled substances (Couri et al., 1977; Toftgard et al., 1981, 1983). BaPOH-ase and 7ECdeEt-ase are associated with the microsomal fraction (Hook et al., 1972; Pelkonen and Vähäkangas, 1980), and in our study the protein content of the microsomal fraction did not change. Conclusions based on specific activity (units/mg protein) may therefore be extrapolated to the whole tissue.

The results of animal studies such as the one described here are difficult

to extrapolate to human exposure (Parke, 1982). They do, however, permit us to experimentally manipulate exposure conditions and thus provide insight into the contribution of occupational exposure to basic detoxication processes. The conditions for the present study were chosen to minimize as much as possible the presence of confounding variables associated with diet, genetic background, and nonmining environments.

The age-related decreases in the Fischer-344 rat liver P-450, BaPOH-ase, and 7ECdeEt-ase activities are similar to the results reported by othe laboratories (Kao and Hudson, 1980; Jayaraj and Richardson, 1981; Schmucker and Wang, 1981). Such decreases may reflect changes in hepatic heme metabolism (Paterniti et al., 1978; Beck et al., 1982), as well as synthesis of new protein (Nebert et al., 1980). Age-dependent changes in hepatic P-450-associated activities are not necessarily reflected in other tissues (McMartin, 1980; Juchan et al., 1979). In our experiments, we observed slight increases in lung P-450 activities as the animals aged.

Other laboratories have reported conflicting results from their studies on the effect of inhaled DE alone on microsomal P-450 activities. When rats were exposed under acute conditions, a slight increase in lung BaPOH-ase activity was observed (Lee et al., 1980). Navarro et al. (1981) and Chen and Vostal (1981), however, reported decreased lung BaPOH-ase activity over 6–12 mo exposure under more chronic conditions. The discrepencies among the results may be due to different exposure regimens. In the present study, animals in the DE chamber were exposed 7 h/d to a total particulate level of 2 mg/m^3 (Green et al., 1983). Lee et al. (1980) used an exposure interval of 20 h/d for 42 d at a higher (6 mg/m^3) particulate level (Tyrer et al., 1980). Navarro et al., (1981) and Chen and Vostal (1981) used chronic exposure conditions similar to ours in terms of total particulate, but their exposure interval was 20 h/d. Not only was our exposure interval about one-third of the former studies, our interval for recovery from daily exposure amounted to four times that allowed in the previous investigation.

During our study of the response of rat lung and liver microsomal P-450 activities to chronic inhalation of CD and/or DE particulates, other rats were similarly exposed and then tested for the presence of dust in tissues and for biological activities. Particulates were observed in lung tissue, although none were seen in liver (Green et al., 1984). Whereas long-term pulmonary dust clearance, resistance to infection, and activation of alveolar macrophages were affected by exposure to CD or DE, short-term clearance and tumor rates were not (Castranova et al., 1984; Green et al., 1984).

Using analysis of variance, only one exposure-related difference was significant. Less lung BaPOH-ase activity was measured in lung microsomes from animals exposed to DECD (specific activity = 2.8) than that measured in rats exposed to CD alone (specific activity = 4.4). There are two possible explanations for the result. (1) An interaction occurred between DE and CD such that the inhaled combination can no longer be considered discrete

entities. (2) The decreased BPOH-ase activity may be indicative of an infectious agent in the DECD-exposed rats (Mannering et al., 1980). Although microscopic examination revealed the presence of more murine pneumonia in the DECD animals than in those exposed to CD alone, the disease was of mild severity. Only a few airways were involved, and the surrounding areas of inflammation were small (F. Green, unpublished results, 1983). Furthermore, clinical symptoms of infection were absent and mortality was less than 1% for all respiratory infections. Sufficient information does not exist at the current time to distinguish between the two possibilities.

It is important to recognize only lung BaPOH-ase activity was changed upon exposure of the animals to DECD compared to that in CD-exposed rats; lung 7ECdeEt-ase, liver 7ECdeEt-ase, and liver BaPOH-ase were unaltered. Furthermore, when lung BaPOH-ase activities from CD- and DECD-exposed rats were independently compared to values obtained from FA, neither differed significantly from the control. The data therefore suggest that exposure to DE and CD, alone or in combination, had little or no effect on the lung and liver microsomal P-450-complex under the conditions of our experiment.

REFERENCES

Beck, W. T., Demon, M. L., and Ovellette, M. S. 1982. Biochemical basis for impaired drug metabolism in tumor-bearing rats: Evidence for altered regulation of hepatic microsomal hemeprotein synthesis. *Biochem. Pharmacol.* 31:1535–1543.

Bend, J. R., Hook, G. E., Easterling, R. E., Gram, T. E., and Fouts, R. J. 1972. A comparative study of the hepatic and pulmonary microsomal mixed-function oxidase systems in the rabbit. *J. Pharmacol. Exp. Ther.* 183:206–217.

Castranova, V., Bowman, L., Reasor, M. J., Lewis, T., Tucker, J., and Miles, P. R. 1984. The response of rat alveolar macrophages to chronic inhalation of coal dust and/or diesel exhaust. *Environ. Res.*, in press.

Chen, K.-C., and Vostal, J. J. 1981. Aryl hydrocarbon hydroxylase activity induced by injected diesel particle extract versus inhalation of diluted diesel exhaust. *J. Appl. Toxicol.* 1:127–131.

Couri, D., Hetland, L. B., Abdel-Rahman, M. S., and Weiss, H. 1977. The influence of inhaled ketone solvent vapors in hepatic microsomal biotransformtation activities. *Toxicol. Appl. Pharmacol.* 41:285–289.

Danner-Rabovsky, J., and Groseclose, R. D. 1982. Stability of rat lung and liver microsomal cytochrome P450 enzyme activities to storage: Purified microsomal fraction, postmitochondrial fraction, and whole tissue. *J. Toxicol. Environ. Health*, 10:601–611.

Estabrook, R. W., Peterson, J., Baron, J., and Hildebrandt, A. 1972. The spectrophotometric measurement of turbid suspensions of cytochromes associated with drug metabolism. *Meth. Pharmacol.* 2:303–350.

Gillette, J. R., Davis, D. C., and Sasame, H. A. 1972. Cytochrome P450 and its role in drug metabolism. *Ann. Rev. Pharmacol.* 12:57–84.

Green, F. H. Y., Boyd, R. L., Danner-Rabovsky, J., Fisher, M. J., Moorman, W. J., Ong, T.-M., Tucker, J., Vallyathan, V., Whong, W.-Z., Zoldak, J., Lewis, T. 1983. Inhalation studies of diesel exhaust and coal dust in rats. *Scand. J. Work, Environ. Health* (Proc. Joint NISOH/Finnish Symp., May 1982, Helsinki, Finland) 9:181–188.

Green, F. H. Y., Hahon, N., Slatkin, D., Obendoerster, Tucker, J., and Lewis, T. R. 1984. Chronic inhalation exposure study of diesel exhaust and coal dust in rodents. *Am. Rev. Respir. Dis.* 129:A139.

Greenlee, W. F., and Pland, A. 1978. An improved assay of 7-ethoxycoumarin-deethylase activity: Induction of hepatic enzyme activity in C57B1/6J and DBA/2J mice by phenobarbital, 3-methycholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Pharmacol. Exp. Ther.* 205:596-605.

Hinners, R. G., Burkart, J. K., Malanchuk, M., and Wagner, W. D. 1980. Facilities for diesel exhaust studies. In *Health Effects of Diesel Engine Emissions: Proceedings of an International Symposium,* Cincinnati, Ohio, December 3-5, 1979, eds. W. E. Pepelko, R. N. Danner, and N. A. Clarke, Vol. 2. pp. 681-697. Cincinnati, Ohio: USEPA.

Hook, G. E. R., Bend, J. R., Hoel, D., Fouts, J. R., and Gran, T. E. 1972. Perparation of lung microsomes and a comparison of the distribution of enzymes between subcellular fractions of rabbit lung and liver. *J. Pharmacol. Exp. Ther.* 182:474-490.

Ioannides, C., and Parke, D. V. 1980. The metabolic activation and detoxication of mutagens and carcinogens. *Chem. Ind. (Lond.)* 21:854-859.

Jayaraj, A., and Richardson, A. 1981. Metabolic activation of aflatoxin B_1 by liver tissue from male Fischer 344 rats of various ages. *Mech. Ageing Dev.* 17:163-171.

Juchau, M. R., DiGiovanni, J., Namkung, M. J., and Jones, A. H. 1979. A comparison of the capacity of fetal and adult liver, lung and brain to convert polycyclic aromatic hydrocarbons to mutagenic and cytotoxic metabolites in mice and rats. *Toxicol. Appl. Pharmacol.* 49:171-178.

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

Kao, J., and Hudson, P. 1980. Induction of hepatic cytochrome P-450 dependent mono-oxygenase system in young and geriatric rats. *Biochem. Pharmacol.* 29:1191-1194.

Lee, I. P., Suzuki, K., Lee, S. D., and Dixon, R. L. 1980. Aryl hydrocarbon hydroxylase induction in rat lung, liver, and male reproductive organs following inhalation exposure to diesel emission. *Toxicol. Appl. Pharmacol.* 52:181-184.

Lowry, O. H., Rosbrough, N. J., Farr, A. L., and Randall, R. J. 1951. Portein measurements with the folin phenol reagent. *J. Biol. Chem.* 193:265-275.

Mannering, G. J. K., Renton, K. W., El Azhary, L., and Deloria, L. B. 1980. Effects of interferon inducing agents on hepatic cytochrome P450 drug metabolizing systems. *Ann. N.Y. Acad. Sci.* 350:314-331.

Matsubara, T., Prough, R. A., Burke, M. D., and Estabrook, R. W. 1974. The preparation of microsomal fracitons of rodent respiratory tract and their characterization. *Cancer Res.* 34:2196-2203.

McMartin, D. D., O'Conner, J. A., Jr., Fasco, M. J., and Kaminsky, L. S. 1980. Influence of aging and induction on rat liver and kidney microsomal mixed function oxidase systems. *Toxicol. Appl. Pharmacol.* 54:411-419.

Minchin, R. F., and Boyd, M. R. 1983. Localization of metabolic activation and deactivation systems in the lung: Significance to the pulmonary toxicity of xenobiotics. *Ann. Rev. Pharmacol. Toxicol.* 23:217-238.

Navarro, C., Charboneau, J., and McCauley, R. 1981. The effect of *in vivo* diesel exhaust on rat hepatic and pulmonary microsomal activities. *J. Appl. Toxicol.* 1:124-126.

Nebert, E. W., and Gelboin, H. V. 1968. Substrate inducible microsomal aryl hydrocarbon hydroxylase in mammalian cell culture. *J. Biol. Chem.* 243:6242-6249.

Nebert, D. W., Jensen, N. M., Perry, J. W., and Oka, T. 1980. Association between ornithine decarboxylase induction and the Ah locus in mice treated with polycyclic aromatic compounds. *J. Biol. Chem.* 255:6836-6842.

Parke, D. V. 1982. Mechanisms of chemical toxicity—A unifying hypothesis. *Reg. Toxicol. Pharmacol.* 2:267-286.

Paterniti, J. R., Jr., Lin, C.-I. P., and Beattie, D. S. 1978. δ-Aminolevulinic acid synthetase: Regulation of activity in various tissues of the aging rat. *Arch. Biochem. Biophys.* 191:792-797.

Pelkonen, O., and Vähäkangas, K. 1980. Carcinogenesis at the molecular level. Part II. Metabolic activation and inactivation of chemical carcinogens. *J. Toxicol. Environ. Health* 6:989-999.

Pepelko, W. E., and Peirano, W. B. 1983. Health effects of exposure to diesel engine emissions. *J. Am. Coll. Toxicol.* 2:253-306.

SAS Institute. 1979. *SAS Users' Guide.* Raleigh, NC: SAS Institute, Inc.

Schmucker, D. L., and Wang, R. K. 1981. Effects of aging and phenobarbital on the rat liver microsomal drug metabolizing system. *Mech. Aging Dev.* 15:189-202.

Searle, S. R. 1971. *Linear Models,* pp. 110-112. New York: Wiley.

Snedecor, G. W., and Cochran, W. G. 1967. *Statistical Methods,* pp. 271-272. Ames, Iowa: Iowa State University Press.

Testa, B., and Jenner, P. 1976. Biochemical Aspects of Drug Metabolism. In *Drugs and the Pharmaceutical Sciences,* vol. 4, pp. 329-408. New York: Marcel Dekker.

Toftgard, R., Nilsen, O. G., and Gustafsson, J.-Å. 1981. Changes in rat liver microsomal cytochrome P450 and enzymatic activities after the inhalation of *n*-hexane, xylene, methyl ethyl ketone and methylchloroform for four weeks. *Scand. J. Work Environ. Health* 7:31-37.

Toftgard, R., Halpert, J., and Gustafsson, J.-Å. 1983. Xylene induces a cytochrome P-450 isozyme in rat liver similar to the major isozyme induced by phenobarbital. *Molec. Pharmacol.* 23:265-271.

Tyrer, H. W., Cantrell, E. T., Horres, R., Lee, I. P., Peirano, W. B., and Danner, R. M. 1980. Benzo[a]-pyrene metabolism in mice exposed to diesel exhaust. I. Uptake and distribution. In *Health Effects of Deisel Engine Emissions: Proceedings of an International Symposium,* Cincinnati, Ohio, December 3-5, 1979, eds. W. E. Pepelko, R. M. Danner, and N. A. Clarke, vol. 1, pp. 508-519. Cincinnati, Ohio: USEPA.

Weibel, F. J., Brown, S., Waters, H. L., and Selkirk, J. K. 1977. Activation of xenobiotics by monooxygenases: Cultures of mammalian cells as analytical tool. *Arch. Toxicol.* 39:133-148.

Received April 28, 1984
Accepted August 3, 1984