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PNEUMOCONIOSIS IN RATS EXPOSED CHRONICALLY TO OIL SHALE DUST AND DIESEL EXHAUST, ALONE AND IN COMBINATION

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<A> ABSTRACT

Rats were exposed by chronic inhalation to diesel exhaust or oil shale dust, alone and in combination to examine pathophysiologic interactions between the dusts. The three agents all accumulated progressively in lungs and caused similar pneumoconiotic responses. The effects of combined exposures tended to be greater than additive. The magnitude of effects was more closely correlated to particle lung burden than to exposure concentration. This suggests that effects of prolonged human exposures to combined dust atmospheres may be estimated better on the basis of predicted lung burden than exposure concentration.

<B> INTRODUCTION

Workers in diesel-powered mines are exposed to combinations of exhaust soot and mineral dusts. The health risk contributed by each agent in a mixed dust exposure, and interactions among agents, are difficult to determine epidemiologically, and few laboratory experiments have examined these issues. Earlier studies demonstrated progressive inflammatory, proliferative, fibrotic, and carcinogenic responses in rats exposed chronically to oil shale dusts (HOLLAND et al., 1986) and diesel exhaust (MAUDERLY et al., 1987a: 1988). The present study examined the effects of 30-month simulated chronic occupational exposures of rats to oil shale dusts and diesel exhaust, singly and in combination, to evaluate the contributions of single agents to pathophysiological effects of mixed exposures.

<C> METHODS

The methods were similar to those used in a short-term study reported previously (MAUDERLY et al., 1987b). Male and female F344/Crl rats from the Institute's colony were conditioned in Hazleton H2000 exposure chambers for 4.5 wk beginning at 15 wk of age. The rats were then exposed 7 hr/day, 5 days/wk for up to 30 mo to raw (RS) or direct-retorted "spent" (SS) oil shale dusts at 5 mg/m^3, to diesel exhaust (DE) at 3.5 mg soot/m^3, or to additive combinations of raw (RSDE) and spent

(SSDE) shale dusts and DE at total particulate concentrations of 8.5 mg/m^3. Control (C) rats were sham-exposed. The rats were housed continuously in the chambers maintained at an airflow of 15 cfm, at 23°C and 40-60% relative humidity, and on a 12-hr light/dark cycle. Feed (Wayne Lab Blox, Allied Mills, Chicago, IL) and water were provided ad libitum.

Raw and direct-retorted "Paraho" shale was obtained from the U.S. Department of Energy Anvil Points mine at Rifle, CO, crushed, ball-milled, and seived through 270-mesh screen, mixed with type 316 stainless steel bed material, and aerosolized using fluid bed generators (CARPENTER AND YERKES, 1980). In the chambers, RS and SS had mass median aerodynamic diameters (± geometric standard deviations) (MMAD, GSD), of 3.1 μm (± 2.0) and 2.9 μm (± 1.9), respectively. The composition of the shale dusts have been reported (MAUDERLY et al., 1987b). Although not determined in this study, the shales were thought to contain 8-12% quartz by mass (HOLLAND et al., 1986). The RS and SS contained 1.4% and 0.03% solvent-extractable organic content, respectively. The DE was generated by 1980 General Motors 5.7L engines burning certification fuel and operated by a computer-dynamometer system on an urban driving cycle (MOKLER et al., 1984). The DE was diluted with air and, in chambers, the soot had an MMAD (± GSD) of 0.25 μm (± 4.5) (CHENG et al., 1984). The solvent-extractable organic content of the DE soot was 12% by mass (BECHTOLD et al., 1984).

Total chamber particle concentrations were measured daily, and exhaust gases and vapors were measured weekly in DE, RSDE, SSDE, and C chambers. The portions of total mass contributed to mixed atmospheres by DE soot and shale dusts were estimated from ratios of exhaust gas concentrations in mixed atmospheres to those in DE.

Lung burdens of particles and health effects in equal numbers of males and females were evaluated at 6-mo intervals; however, the present report includes only data collected at the end of the exposures. Lung burdens of shale dusts were determined by atomic absorption analysis of aluminum, and lung burdens of DE soot were determined from light absorption by homogenates (MAUDERLY et al., 1986). Fluid recovered by bronchoalveolar lavage of 8 rats/group with saline was analyzed for cells, cytoplasmic and lysosomal enzymes, collagenous peptides, and total protein; total lung tissue collagen was also measured (HENDERSON et al., 1988). The respiratory function of 12 rats/group was

measured by plethysmography (MAUDERLY et al., 1988). The clearance rate of tracer particles was measured by exposing 12 rats/group by inhalation to 0.1 μm iron-59 oxide particles after 18 mo of chronic exposure, followed by whole-body counting of radioactivity during the subsequent 6 mo of continued chronic exposure. The lungs of 8 rats/group were weighed and fixed by constant-pressure perfusion. Excised lung volume was measured by water displacement, and sections were stained for evaluation of histopathology by light microscopy.

<D> **RESULTS**

Exposure concentrations and lung burdens of particles are listed in Table 1. Gas and vapor concentrations are not listed because it has been shown that filtered DE does not cause pneumoconiosis in rats (HEINRICH et al., 1986). For reference, however, carbon monoxide = 12.9 ppm, nitrogen oxides = 4.6 ppm, and hydrocarbon vapor = 3.1 ppm for DE. Four to 5 times more DE soot than shale dust accumulated in the lung per unit of exposure concentration in rats exposed to single agents, partially due to particle size. The pulmonary deposition fraction of DE would be approximately twice those of RS and SS (SNIPES, 1989), but the normal clearance rate of the three materials would be similar (SNIPES et al., 1983). In the mixed exposures, the lung burden-exposure concentration ratios of shale dusts were similar to those in single exposures, while those of DE were slightly higher ($P<0.06$ for soot in RSDE vs. DE). This suggests that mixed exposures very slightly enhanced the accumulation of DE soot, but not that of shale dust. <Table 1 goes here>

Body weight and survival were not significantly affected. Progressive pneumoconiotic responses occurred in all exposed groups, paralleling the accumulation of dusts. Particles were contained largely in macrophages, which formed focal aggregates. The focal accumulations were accompanied by chronic, active inflammation and widespread focal epithelial hyperplasia, metaplasia, and fibrosis as reported previously for chronic shale and DE exposures (HOLLAND et al., 1986; MAUDERLY et al., 1988; MAUDERLY et al., 1990). The histopathological changes were qualitatively similar in all exposed groups, but greater in magnitude in groups receiving combined exposures.

Table 2 contains representative data illustrating key features of the effects of exposure, which were qualitatively similar for all exposed groups, but differed in magnitude. Excised lung weight and density were increased. In bronchoalveolar lavage fluid, increased leukocytes demonstrated

inflammation, and increases in cytoplasmic (lactate dehydrogenase) and lysosomal (acid proteinase) enzymes reflected cell damage and increased phagocytosis and phagocytic cell death, respectively. Lung collagen was increased (not shown) in direct proportion to lung weight. A restrictive respiratory function impairment was reflected by reduced lung volumes (total lung capacity), increased lung stiffness (compliance), and reduced alveolar-capillary gas exchange efficiency (CO diffusing capacity). There was no significant airflow obstruction. The half-time of long-term particle clearance was increased significantly by all exposures. <Table 2 goes here>

Figure 1 illustrates the relative magnitudes of the above effects among exposed groups. Data in Table 2 were expressed as percentage differences from control values, regardless of sign (negative or positive differences), and the parameters listed under each of the four categories in Table 2 were combined into single mean differences for each category. The combined effects of exposure to DE and shale dusts were clearly greater than additive for lung weight and density and for lavage parameters, and were slightly greater than additive for respiratory function. Effects of combined exposures were less than additive for clearance half-time. <Figure 1 goes here>

Figure 2 illustrates the relative magnitudes of the above effects among exposed groups, combining the mean of all nine parameters in Table 2 into a single index of "total effects". The total effects of combined exposures to DE and shale dusts were greater than additive. The total effects normalized by exposure concentration were less than additive. The value for DE alone was similar to those for combined exposures, but much lower values were obtained for RS and SS. The total effects normalized by lung burden were somewhat more similar among the groups. The total effects/lung burden were identical in magnitude for DE and RS, nearly 50% greater for SS, and slightly higher for combined than for single exposures. <Figure 2 goes here)

<E> CONCLUSIONS

The SS was slightly more toxic per unit of exposure concentration or lung burden than RS in the rat. Although unconfirmed, this might have resulted from a difference in specific surface area, or surface characteristics between RS and SS due to retorting. The extrapolation of the results of this study to man relies on the assumption that human and rat lungs would respond similarly if similarly loaded with dusts. If so, these findings suggest that in prolonged exposures to mixed atmospheres

of DE and shale dust, DE soot may accumulate more rapidly than shale dust in the lung and may dominate the adverse effects. Although the effects of exposure to DE and shale dust were greater than additive, the effects could largely be normalized on the basis of the total accumulated lung burden of particles. This suggests that the effects of human exposures to mixed dusts of these types are better estimated on the basis of predicted total particle lung burdens than on the basis of exposure concentration.

<F> **ACKNOWLEDGEMENT**

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FIGURE LEGENDS

Figure 1. The magnitudes of the four categories of effects listed in Table 2 are compared among exposure groups as percentage differences from control values. Values for multiple parameters were averaged for each of the first three categories.

Figure 2. The average magnitudes of all nine parameters listed in Table 2 are compared among exposure groups as mean percentage differences from control values, percentage differences divided by particle exposure concentrations, and percentage differences divided by particle lung burdens.

Table 1. Relationship of Particle Lung Burden to Exposure Concentration After 30 Months of Exposure[a]

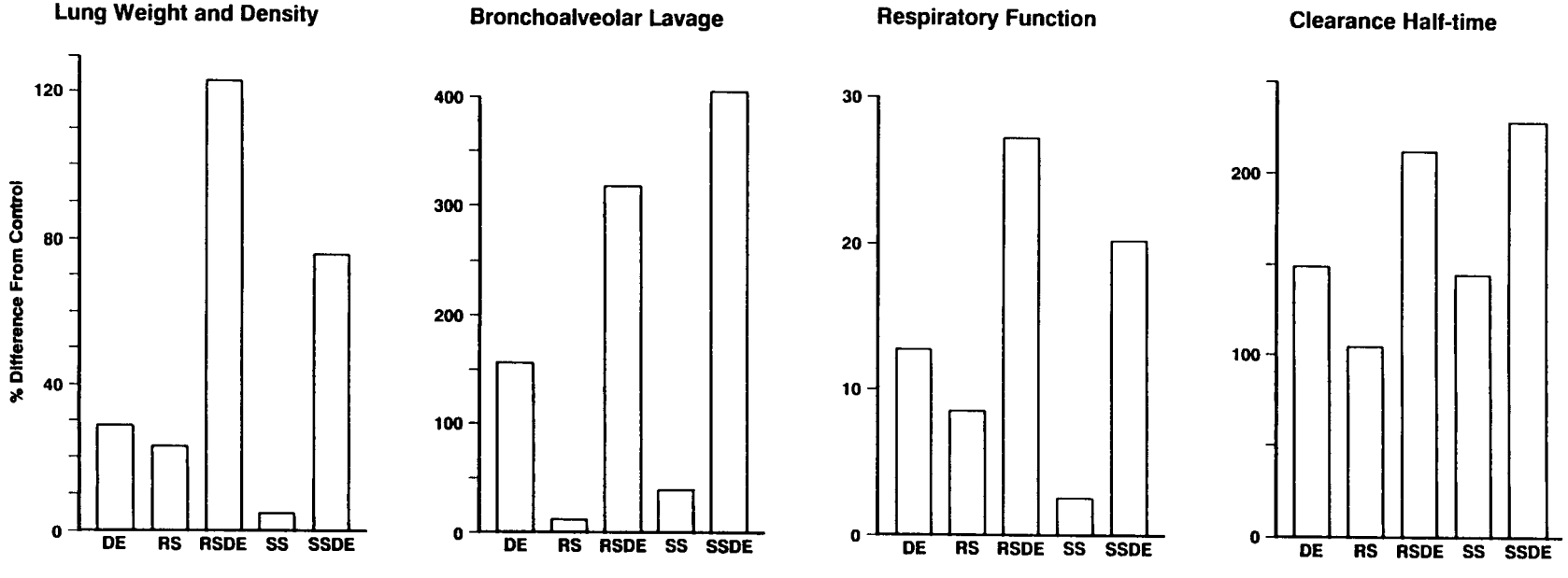
	Diesel Soot		Shale Dust		Total	
Group	Mean	SD	Mean	SD	Mean	SD
Exposure Concentration (mg/m^3)						
DE	3.5	0.2	0		3.5	0.2
RS	0		5.1	0.7	5.1	0.7
RSDE	3.6[b]		4.9[b]		8.5	0.5
SS	0		5.0	0.5	5.0	0.5
SSDE	3.4[b]		5.2[b]		8.6	0.6
C	0		0		0.05	0.02
Lung Burden (mg)						
DE	14.7	1.5	0		14.7	1.5
RS	0		4.5	1.5	4.5	1.5
RSDE	18.4	4.2	4.4	0.9	22.8	4.8
SS	0		3.9	1.0	3.9	1.0
SSDE	15.5	3.3	3.8	1.4	19.3	3.3
Lung Burden/Exposure Concentration ($mg/mg/m^3$)						
DE	4.20	0.44	0		4.20	0.44
RS	0		0.88	0.29	0.88	0.29
RSDE	5.12	1.17	0.89	0.18	2.68	0.56
SS	0		0.77	0.21	0.77	0.21
SSDE	4.56	0.97	0.72	0.27	2.24	0.38

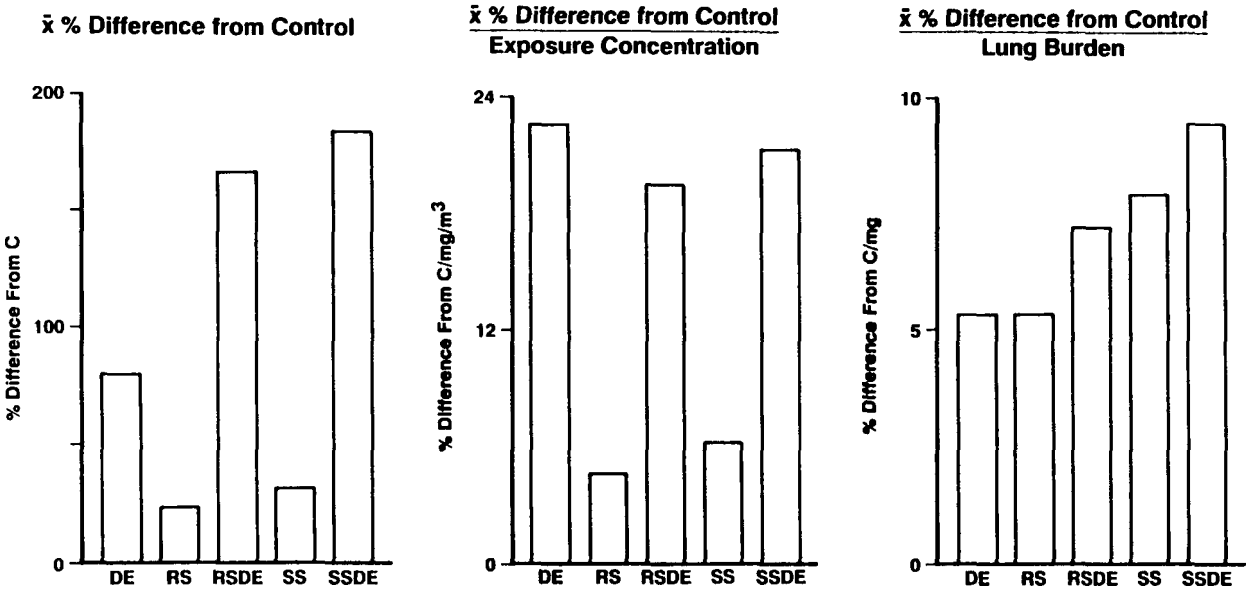
[a]Exposure data are means of weekly mean values for 30 mo. Lung burden data were measured after 30 mo of exposure.
[b]Mean values estimated from ratio of exhaust gas concentrations to those in DE atmosphere.

Table 2. Representative Effects of 30 Months of Exposure

		C		DE		RS		RSDE		SS		SSDE	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lung Weight and Density													
Lung Weight/Body Weight	g/kg	7.3	0.7	8.9	0.7	8.7	0.7	13.5	1.2	6.7	0.8	11.1	1.0
Lung Density	g/ml	0.11	0.01	0.15[a]	0.01	0.14	0.01	0.29a	0.04	0.13	0.02	0.22[a]	0.02
Bronchoalveolar Lavage													
Total Leukocytes	10^6/ml	1.5	0.4	2.7	0.4	1.6	0.1	5.5	1.6	1.9	0.2	9.5[a]	1.1
Lactate Dehydrogenase	mIU/ml	81	14	255[a]	36	65	9	456[a]	79	99	14	446[a]	51
Acid Proteinase	mgHb/hr/ml	32	2	88[a]	5	49[a]	3	105[a]	3	55[a]	3	107[a]	3
Respiratory Function													
Total Lung Capacity	ml	16.3	1.3	14.4	1.4	15.4	1.1	12.4	1.1	15.8	0.6	13.1	1.2
Quasistatic Chord Compliance	ml/cmH_2O	0.92	0.07	0.81	0.10	0.84	0.05	0.59[a]	0.06	0.88	0.04	0.70	0.10
CO Diffusing Capacity	ml/min/mmHg	0.188	0.013	0.161	0.019	0.166	0.019	0.147	0.021	0.188	0.020	0.156	0.015
Clearance of Tracer Particles $T_{1/2}$ of Long-Term Component	days	61	5	152[a]	31	125[a]	19	190[a]	49	149[a]	15	200[a]	51

[a]Difference from C significant at $P < 0.05$ by t-test and adjustment for multiple comparison.


Lung Weight and Density
% Difference From Control
120
80
40
0
DE
RS
RSDE
SS
SSDE
Bronchoalveolar Lavage
400
300
200
100
0
DE
RS
RSDE
SS
SSDE
Respiratory Function
30
20
10
0
DE
RS
RSDE
SS
SSDE
Clearance Half-time
200
100
0
DE
RS
RSDE
SS
SSDE


x̄ % Difference from Control
% Difference From C
200
100
0
DE
RS
RSDE
SS
SSDE
x̄ % Difference from Control
Exposure Concentration
% Difference From C/mg/m3
24
12
0
DE
RS
RSDE
SS
SSDE
x̄ % Difference from Control
Lung Burden
% Difference From C/mg
10
5
0
DE
RS
RSDE
SS
SSDE

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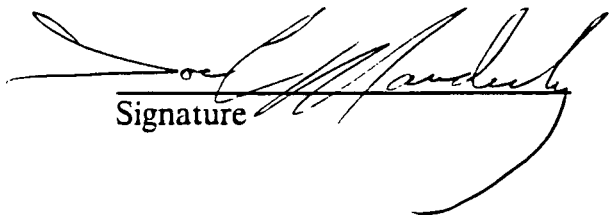

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