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To cite this article: Lloyd E. Stettler , Jim E. Proctor , S. Frank Platek , Robert J. Carolan , Randall J. Smith & Harry M. Donaldson (1988) Fibrogenicity and carcinogenic potential of smelter slags used as abrasive blasting substitutes, Journal of Toxicology and Environmental Health, Part A Current Issues, 25:1, 35-56, DOI: [10.1080/15287398809531187](https://doi.org/10.1080/15287398809531187)

To link to this article: <https://doi.org/10.1080/15287398809531187>



Published online: 19 Oct 2009.



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FIBROGENICITY AND CARCINOGENIC POTENTIAL OF SMELTER SLAGS USED AS ABRASIVE BLASTING SUBSTITUTES

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This study was designed to examine the fibrogenic and carcinogenic potentials of three smelter slags (primary copper slag, secondary copper slag, and nickel slag) that have been used for a number of years as substitutes for sand in abrasive blasting operations. Seven groups of 85 male Fischer 344 rats (approximately 180 g) were used. Each group was given a single 20-mg dose of one of the following test materials via intratracheal instillation: primary copper slag, secondary copper slag, nickel slag, feldspar, Min-U-Sil, novaculite, or vehicle control. Chemical, particle size, and surface area analyses were performed for each test dust. Animals were weighed monthly, and ten animals per group were necropsied at the 6-, 12-, and 18-mo interim sacrifices. The terminal sacrifice was conducted at 22 mo. Hematoxylin and eosin stained histologic sections were prepared from designated formalin-fixed tissues collected at necropsy and examined microscopically.

The pulmonary fibrogenic and carcinogenic potentials of the three smelter slags were compared histopathologically with feldspar, novaculite, Min-U-Sil, and vehicle controls. Only minimal to slight alveolar wall fibrosis was seen in the two copper slag groups, while the response seen with nickel slag was consistent with a foreign body

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reaction with minimal fibrosis seen in only an occasional animal. The major reaction seen in both the feldspar- and the novaculite-treated rats was a granulomatous inflammation with varying degrees of fibrosis associated with the granulomas.

Significant numbers of primary lung tumors, principally adenocarcinomas and adenomas, were seen in the copper slag ($p = 0.005$ and $p = 0.022$ for the primary and secondary slags, respectively), in the feldspar ($p = 0.007$), in the novaculite ($p < 0.001$), and in the Min-U-Sil ($p < 0.001$) groups when compared to the vehicle control group. In addition, the Min-U-Sil and novaculite groups had significantly elevated pulmonary tumor proportions relative to the other treatments ($p \leq 0.002$), with the Min-U-Sil being higher than the novaculite ($p = 0.012$). On the basis of the tumor incidence data, one must conclude that both copper slags tested in this study are carcinogenic to rats.

INTRODUCTION

For several years our laboratory has been involved in the biological testing of materials that have been suggested as, or are being used as, substitutes for other agents. This work has primarily been with coal and smelter slags being used as substitutes for silica sand in abrasive blasting operations. In one study (Mackay et al., 1980), rats were treated by intratracheal instillation with either a coal slag or one of two samples of copper slags. Pulmonary fibrosis was seen in the coal slag-treated rats, while no fibrosis was seen in either copper slag treatment group. Granulomas were seen in the lungs of all treatment groups.

In a second study (Stettler et al., 1982), the trace element contents of 18 slags were determined. Varying amounts of suspect carcinogens such as beryllium, chromium, arsenic, and nickel were found in the analyzed slags. In general, the highest concentrations of suspect carcinogens were found in the smelter slags. These analyses suggested that such slags may be carcinogenic. Consequently, a second animal study was initiated to determine the fibrogenic and/or carcinogenic potentials of three of the smelter slags containing the highest levels of suspected carcinogens. The final results of this second animal study will be presented in this report. A preliminary report discussing interim results of this study has been published elsewhere (Stettler et al., 1983).

In addition to the smelter slags, three other particulate materials were tested in this study. These were novaculite, a finely divided microcrystalline form of quartz; a feldspar, specifically bytownite, a member of the anorthosite group of plagioclase feldspar rock minerals; and Min-U-Sil quartz, which was used as a positive control for fibrosis for the other test agents. Novaculite is used as a pigment and extender in plastics, paint systems, and coatings; as a filler for wood and silicone rubber products; and as an abrasive in metal polishing. Our interest in novaculite was its potential as a positive control for pulmonary fibrosis in animal studies. The anorthosite group of plagioclase feldspar miner-

als is the largest single aluminum resource in the United States (Barclay and Peters, 1976) and is considered as the future primary source of aluminum, replacing bauxite ore. A report summarizing the results of the novaculite and Min-U-Sil test groups has been published elsewhere (Groth et al., 1986).

MATERIALS AND METHODS

Dust Preparation and Characterization

Six dust samples were tested in this study. The first dust was a commercial grit slag sample whose source was a secondary copper smelter, i.e., a scrap metal smelter. Two other test slags were obtained directly from primary smelters, a copper ore smelter and a nickel ore smelter. The feldspar tested was a sample of bytownite obtained from Ward's Natural Science Establishment, Rochester, N.Y. The novaculite sample was obtained from Malvern Minerals Co., Hot Springs, Ark. The sixth test material was a sample of 5- μ m Min-U-Sil obtained from the Pennsylvania Glass Sand Corporation, Pittsburgh, Pa.

The particle sizes of the three slag samples and the feldspar sample were reduced by air-jet milling using a Trost mill (Gem T Research Model, Garlock, Inc.). The samples of novaculite and Min-U-Sil were used as supplied by the manufacturer. All six dust samples used in the subsequent animal exposure tests were sized with a scanning electron microscope (model JXA-50A, JEOL USA, Inc.) equipped with an image analysis system (model B-10, LeMont Scientific). The particle size distributions are summarized in Table 1. Surface areas for each of the test agents were determined by nitrogen adsorption using an automatic sur-

TABLE 1. Dust Particle Size Distributions and Surface Area

Size range of test materials (μ m)	Secondary copper slag (%)	Primary copper slag (%)	Nickel slag (%)	Feldspar (%)	Novaculite (%)	Min-U-Sil (%)
<0.5	41.0	28.0	31.0	27.4	38.4	41.3
0.5-<1.0	12.9	9.0	12.0	17.8	27.3	31.4
1.0-<1.5	7.8	6.6	8.6	12.0	11.7	12.7
1.5-<2.0	8.3	12.3	12.5	9.3	6.3	7.4
2.0-<3.0	11.5	22.2	14.4	13.8	7.3	5.4
3.0-<5.0	11.8	16.6	12.6	14.3	6.8	1.6
5.0-<10.0	5.8	5.2	7.3	5.3	2.2	0.1
>10.0	0.7	0.3	1.5	0.1	0	0
Surface area (m^2/g)	0.8	1.6	0.6	3.3	1.6	4.3

face area analyzer (model 2200, Micromeritics). The dust surface areas are also summarized in Table 1. Chemical analyses were performed for each sample by proton-induced X-ray emission (PIXE) and atomic absorption. The slag samples are glassy silicates whose major components are varying amounts of sodium, magnesium, aluminium, potassium, calcium, and iron. The major components of the feldspar sample are sodium, calcium, aluminium, and silicon. The Min-U-Sil and novaculite samples are quartz. The trace element chemistry of the six test materials is given in Table 2. As can be seen in Table 2, the suspect carcinogen content of the slags is quite varied. Beryllium, chromium, and nickel are found in abundance in the secondary copper slag, while arsenic is a significant component of the primary copper slag. The nickel slag contains relatively large amounts of both nickel and chromium.

Animal Study

The test animals for this study were male, cesarian-derived, Fischer 344 rats (Charles River Breeding Laboratories, Inc.). These rats, as received, had body weights of 180 ± 15 g. After a 2-wk quarantine period, the rats were randomly divided into 7 equal treatment groups of 85 rats each. The rats were housed in a single room nominally maintained at $72 \pm 5^\circ\text{F}$ and $50 \pm 5\%$ relative humidity with a 12-h dark and light cycle. Rats within a group were housed three per cage, fed a commercial pelleted diet (Purina Rat Chow), and given tap water ad libitum for the duration of the study.

The rats in the 6 dust treatment groups were given a single 20-mg intratracheal instillation of the appropriate test agent. The procedure used in these instillations has been described elsewhere (Mackay et al., 1980). The rats in the seventh treatment group were given an instillation of the vehicle used to suspend the treatment dusts (filtered, deionized water).

Following the administration of the test materials, the rats were held for a period of 22 mo. During this period, they were weighed on a monthly basis. Rat necropsy intervals and on-study mortality are indicated in Table 3. For scheduled necropsies, the rats were killed with an overdose of sodium pentobarbital administered by intraperitoneal injection. The lungs were weighed and then inflated with 10% neutral, phosphate-buffered formalin. Sections of the following organs were also fixed in 10% neutral, phosphate-buffered formalin: brain, pituitary, heart, sternum, liver, tracheobronchial lymph nodes, thyroid, parathyroid, spleen, kidneys, testes, prostate, pancreas, and adrenals. Hematoxylin and eosin-stained sections of each of the above tissues were prepared for, and examined by, light microscopy.

TABLE 2. Trace Analyses of Test Materials ($\mu\text{g/g}$)

Element	Secondary copper slag	Primary copper slag	Nickel slag	Feldspar	Novaculite	Min-U-Sil
Be	180 \pm 10	<6	<6	<1.5	<0.4	1.1 \pm 0.4
Ti	1500 \pm 100	1800 \pm 300	250 \pm 70	160 \pm 40	370 \pm 20	100 \pm 20
V	<80	<180	<60	<30	<17	<20
Cr	2400 \pm 100	340 \pm 90	3700 \pm 200	81 \pm 9	<10	<14
Mn	1400 \pm 200	<400	1100 \pm 100	90 \pm 10	<13	<11
Ni	2240 \pm 70	<20	1400 \pm 100	16 \pm 5	6 \pm 2	<4
Cu	5000 \pm 200	4200 \pm 400	17 \pm 2	7 \pm 2	<2	<2
Zn	18900 \pm 500	26000 \pm 2000	75 \pm 2	9 \pm 1	14 \pm 3	11.7 \pm 0.7
As	<40	1450 \pm 60	<2	17 \pm 2	<1.2	<1.1
Se	18 \pm 5	<8	<2	<1.7	<0.7	<1
Br	<12	<10	5 \pm 1	80 \pm 5	5 \pm 1	11 \pm 1
Rb	<6	<12	<2	2.4 \pm 0.9	1.5 \pm 0.3	<1
Sr	77 \pm 5	151 \pm 10	5 \pm 1	390 \pm 20	80 \pm 10	10.5 \pm 0.5
Sc	<400	500 \pm 100	<200	800 \pm 100	130 \pm 40	<140
Y	<20	<8	<2	<1.8	68 \pm 4	2.6 \pm 0.4
Zr	264 \pm 8	69 \pm 6	15 \pm 2	<5	18.8 \pm 0.9	19 \pm 1
Nb	19 \pm 5	<6	<2	<1.8	2.7 \pm 0.6	<0.9
Mo	160 \pm 20	480 \pm 1j0	<2	<2	<1.3	<2
Sn	1260 \pm 30	60 \pm 10	15 \pm 5	<13	<7	<9
Sb	<20	500 \pm 40	<12	<16	<11	<13
Ba	700 \pm 200	<80	<60	<50	150 \pm 10	<30
Pb	8900 \pm 100	1200 \pm 70	<4	<6	17 \pm 2	5 \pm 2

TABLE 3. Animal Disposition and Tumor Incidence

Treatment group	Died 0-6 mo	6 mo Sacrifice	Died 6-12 mo	12-mo Sacrifice	Died 12-18 mo	18-mo Sacrifice	Died 18-22 mo	22-mo Sacrifice
Primary copper slag	0	0/10 ^a	0	2/10	1/3	0/10	0/5	7/47
Secondary copper slag	0	0/10	0 ^b	1/10	0/2	0/10	0/9	6/43
Nickel slag	0	0/10	0	0/10	1/5 ^c	1/9 ^c	0/11	0/40
Min-U-Sil	0	0/10	0/7	1/10	0/5 ^d	3/10	3/12	23/30
Novaculite	0	0/10	0/2	1/10	0/8	2/10	3/11	14/34
Feldspar	0	0/10	0	1/10	0/4	2/10	1/9	5/42
Vehicle control	0	0/10	0	0/10	0/2	0/10	0/10	1/43

^aThe number of animals with lung tumors is in the numerator and the total number of animals examined is in the denominator.

^bOne rat died during this period but no histopathology was performed.

^cOne rat died in transit during the 18-mo sacrifice and was treated as a spontaneous death in the statistical analyses.

^dOne rat was lost during this period and is not reflected here.

RESULTS

Body weight curves for the seven test groups are shown in Figs. 1 and 2. As can be seen from these figures, the body weights for the Min-U-Sil group were much lower than for the other test groups. Survival rates for the Min-U-Sil ($p < 0.001$) and novaculite ($p = 0.024$) groups were significantly reduced when compared to the vehicle control group as determined by the Gehan test (Miller, 1981). The rats from the other treatment groups had survival rates similar to the control group.

Data on the lung weights at the terminal sacrifice are given in Table 4. The lung weights for the Min-U-Sil, novaculite, feldspar, and the primary and secondary copper slags were significantly larger than the vehicle controls ($p < 0.001$), as were the lung weights of the nickel slag exposure group ($p = 0.015$), using two-tailed Van der Waerden (non-parametric) tests (Lehmann, 1975).

Pathology

For all treatment groups, the target organs were the lungs, trachea, and tracheobronchial lymph nodes. Treatment-related lesions were not evident in other tissues. The major histopathological findings for each treatment group are summarized below. The severity of the lesions were graded as follows: minimal, slight, moderate, moderately severe, and severe. The neoplasms observed in the lungs for the seven treatment groups are summarized in Table 3.

Vehicle Controls

Only one primary lung tumor, an adenocarcinoma in a rat necropsied at 22 mo, was seen in the vehicle control rats.

At 6 mo, minimal to moderate perivascular and peribronchial mono-

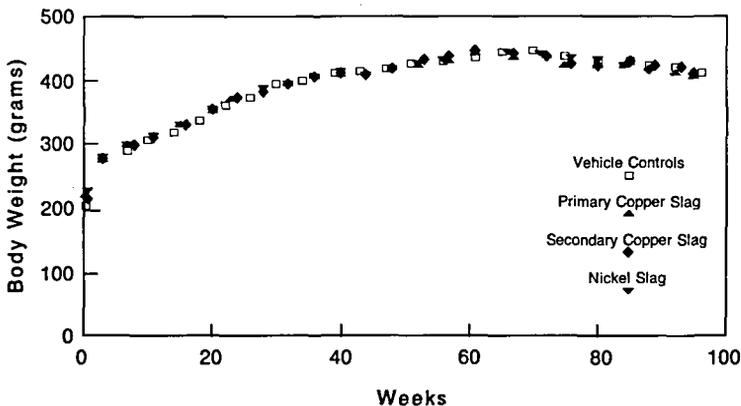


FIGURE 1. Average body weights for the vehicle control and metal slag exposure groups.

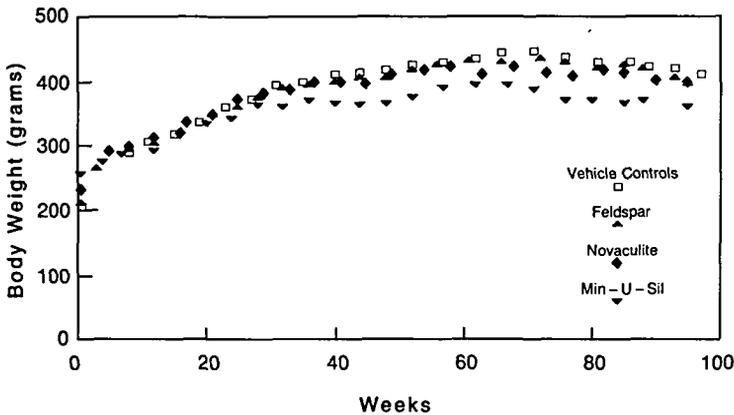


FIGURE 2. Average body weights for the vehicle control and mineral exposure groups.

nuclear infiltrates were present in the lungs of all sacrificed rats. Minimal to slight focal pneumonia was scattered throughout the lungs. These mild lesions in the lungs are suggestive of exposure to murine respiratory pathogens. Incidental pulmonary changes observed in the rat lungs at 12 mo were similar to that observed at 6 mo. The severity of the peribronchial and perivascular mononuclear infiltrates was minimal. Minimal chronic bronchitis was noted in 1 rat and a minimal number of pulmonary macrophages were noted in 2 others at 12 mo. Hemosiderin-like pigment was noted in a tracheobronchial lymph node of 1 rat at the 12-mo sacrifice.

At 18 mo, peribronchial mononuclear infiltrates were evident in most of the lungs, but the minimal changes noted earlier such as perivascular mononuclear infiltrates, chronic bronchitis, etc., were absent. Only one rat had minimal focal pneumonia. Hemosiderin-like pigment

TABLE 4. Descriptive Statistics and Nonparametric Hypotheses Tests Using Data on Lung Weights of Rats Undergoing Terminal Sacrifice

Treatment group	Sample size	Lung weights (g)			Van der Waerden test, treatment vs. control		
		Mean	Standard deviation	Median	Chi-square	df	Pr > Chi-square
Primary copper slag	47	2.64	0.71	2.42	16.18	1	<0.001
Secondary copper slag	41	2.90	1.11	2.62	21.72	1	<0.001
Nickel slag	40	2.37	0.41	2.28	5.93	1	0.015
Min-U-Sil	30	6.70	2.01	6.30	45.28	1	<0.001
Novaculite	34	4.11	1.07	3.78	44.89	1	<0.001
Feldspar	40	2.66	0.54	2.50	19.02	1	<0.001
Vehicle control	42	2.21	0.55	2.09			

(seven animals) and hemorrhage (three animals) were noted in the tracheobronchial lymph nodes.

At 22 mo, chronic pulmonary changes consisted of perivascular and peribronchial mononuclear infiltrates ranging from minimal to moderately severe. Scattered alveolar macrophages were present in most of the lungs. The following lung lesions, commonly present in aged rats, were also noted: type II epithelial hyperplasia in 1 lung, chronic bronchitis ranging from moderate to moderately severe in 2 lungs, minimal to slight focal pneumonia in 13 lungs, and an abscess in 1 lung. Chronic tracheitis ranging from slight to moderately severe was noted in eight animals. Hemosiderin-like pigment (21 animals) and hemorrhage (10 animals) were noted in the tracheobronchial lymph nodes.

The pneumonia and perivascular mononuclear infiltrates observed at each scheduled necropsy interval are frequently observed in rats housed conventionally in which there has been exposure to respiratory pathogens.

Copper Slags

The histopathologic findings for the primary copper slag and the secondary copper slag treatment groups are essentially the same, and are considered together in the following discussion.

Ten primary copper slag-treated rats were found to have lung tumors as shown in Table 3. All of these tumors were adenomas. Multiple adenomas were not observed in any animals. Primary lung tumors were detected in seven animals treated with the secondary copper slag with no lungs having multiple tumors. Primary adenomas were found in 1 rat at 12 mo (Fig. 3) and in 4 rats at 22 mo. Two adenocarcinomas were seen at 22 mo (Fig. 4).

The lung tissues from the rats receiving copper slags were qualitatively similar to each other at all sacrifice intervals. Chronic pulmonary lesions in both groups were typical for pneumoconiosis. In addition to the changes noted in the vehicle control group, pigment-containing macrophages were present in the alveoli of most animals. Type II epithelial hyperplasia and minimal to slight alveolar wall fibrosis were present in scattered alveolar walls adjacent to the pigmented materials. The number of pigment-containing macrophages and the amount of pigmented material in the lung gradually decreased during the course of the experiment. This was particularly evident when lungs from the 6-mo sacrifices were compared with the 22-mo sacrifice animals. A concomitant decrease in Type II epithelial hyperplasia and alveolar wall fibrosis was also noted when 6- and 22-mo lungs were compared, suggesting lung clearance and recovery.

Chronic bronchitis in both copper slag treatment groups was more severe and present in more animals at the 22-mo sacrifice than at earlier sacrifice periods. Chronic tracheitis was also a more common finding at

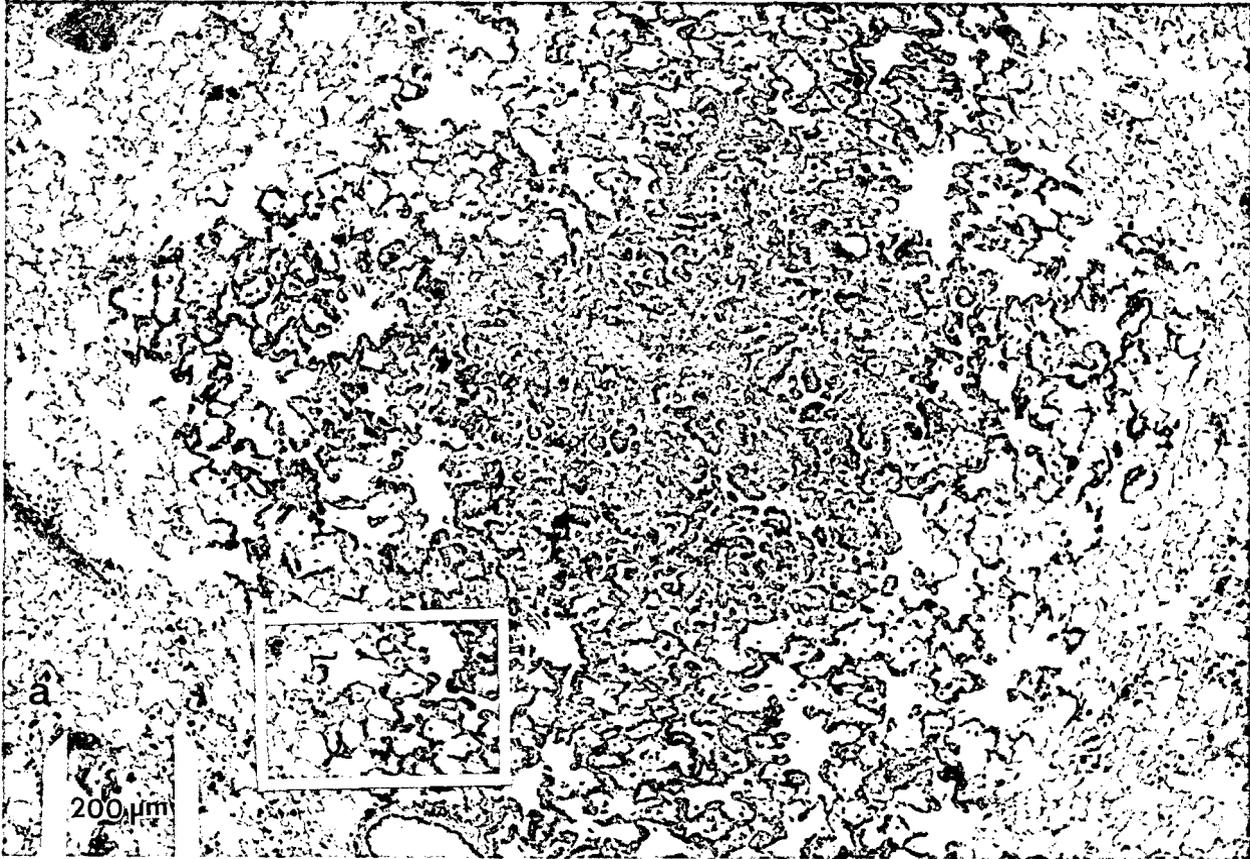


FIGURE 3. (a) Light micrograph of a 5- μm -thick H&E-stained section of lung from a secondary copper slag-treated rat at 12 mo showing a discrete adenoma. Aggregates of pigmented macrophages are randomly scattered throughout.

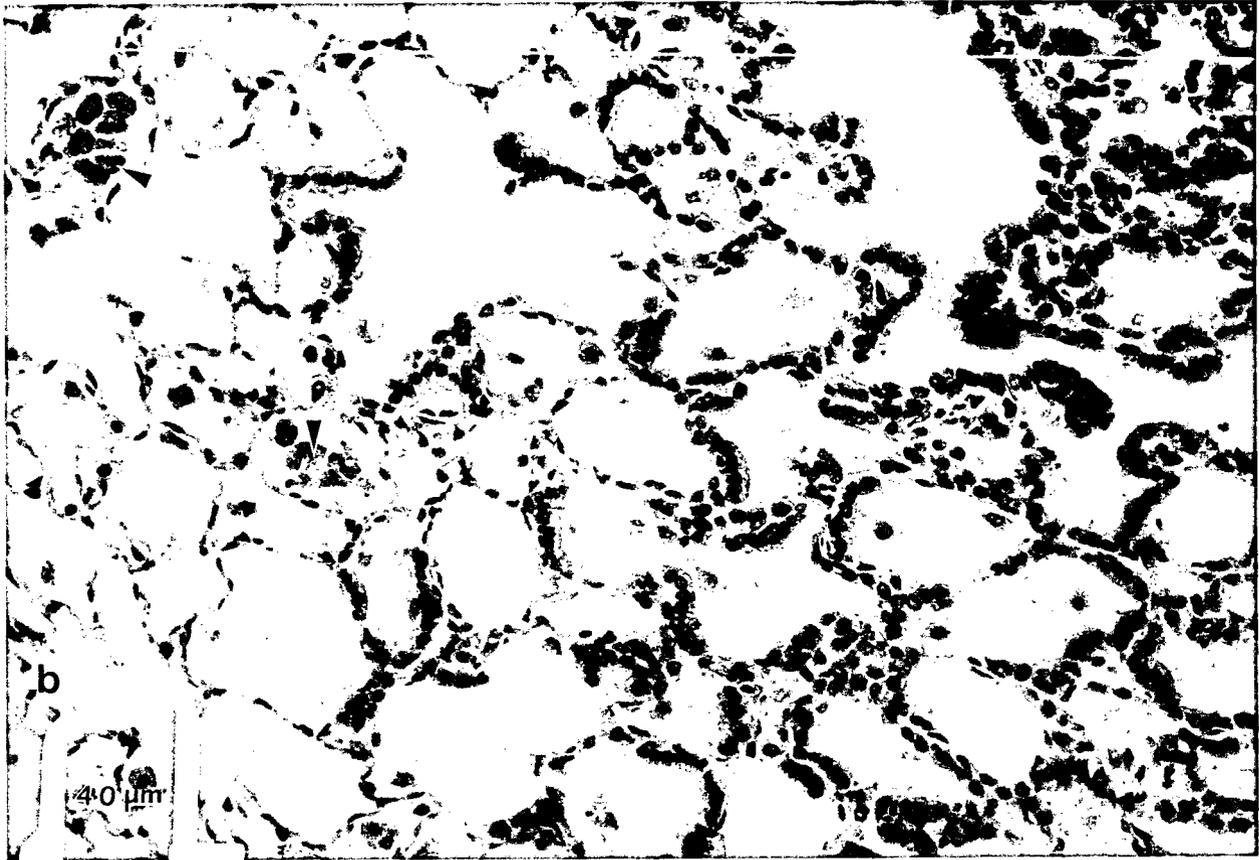


FIGURE 3. (Continued) (b) Enlargement of the inset in (a). Well-differentiated epithelial cells line alveolar structures near the tumor margin. Arrows denote pigmented alveolar macrophages.

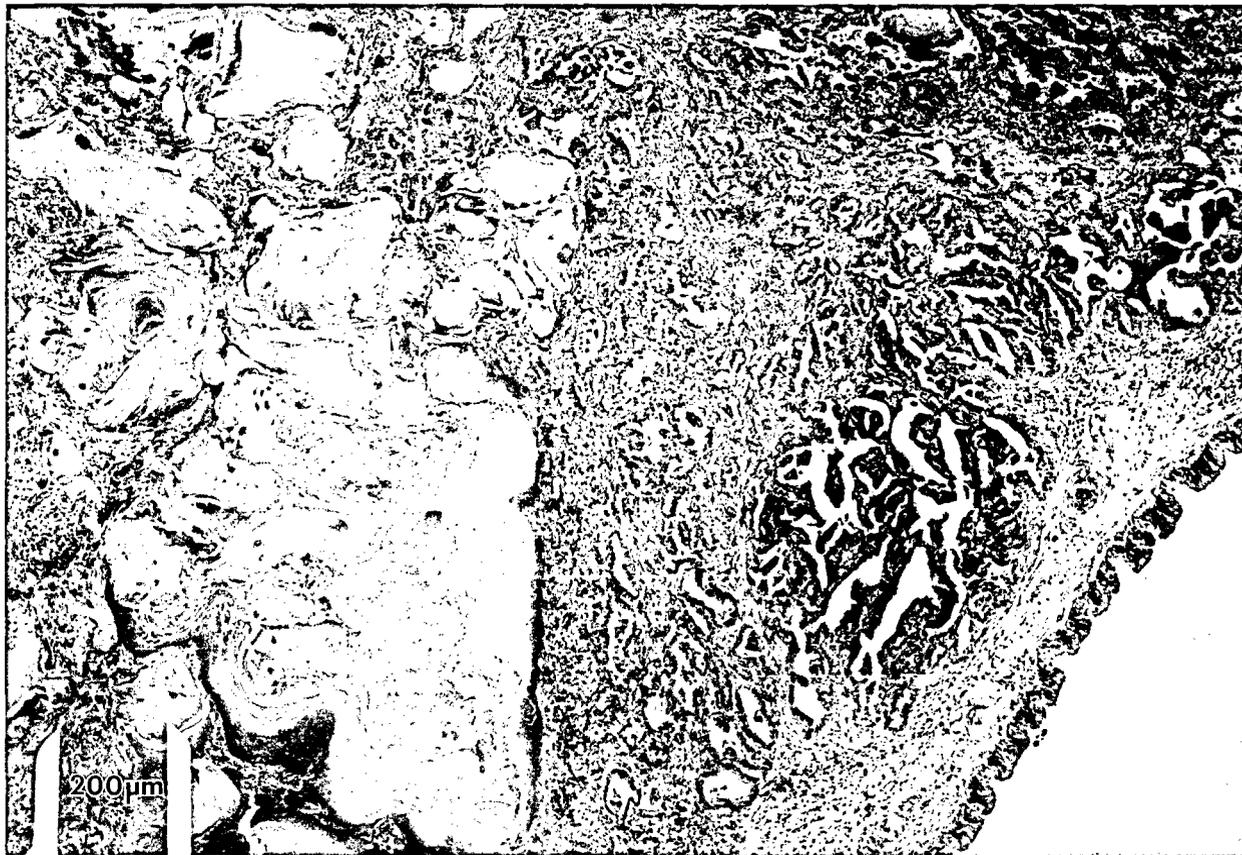


FIGURE 4. Light micrograph of a 5- μm -thick H&E-stained section of lung from a secondary copper slag-treated rat at 22 mo showing an adenocarcinoma. The left half of the photo shows abundant squamous change within the neoplasm.

22 mo than at earlier sacrifice periods for both copper slag treatment groups. Pigment similar to that seen in the lungs was present in macrophages of the tracheobronchial lymph nodes of rats at all sacrifice periods for both copper slags. There was, however, no secondary inflammatory reaction present around this pigment in the lymph nodes.

Nickel Slag

Only two rats treated with nickel slag were found to have primary lung tumors. The lung of 1 rat sacrificed at 18 mo had multiple adenomas. One of 4 rats that died between 12 and 18 mo had a single primary adenoma.

The most notable pulmonary lesion produced by nickel slag consisted of multifocal, variably sized aggregates of pigmented macrophages within the alveoli. Type II epithelial hyperplasia and alveolar wall fibrosis were minimal and were seen only in an occasional animal at the various sacrifice intervals. The number of macrophages in the alveoli and the amount of the anisotropic pigment in the lungs decreased with time during the experiment.

As with the copper slags, chronic bronchitis and chronic tracheitis were more severe and present in more animals at 22 mo than at earlier sacrifice periods. Pigment in tracheobronchial lymph nodes was similar to that seen in the alveolar macrophages.

Feldspar

Nine feldspar-treated rats were found to have pulmonary neoplasms. An adenoma was present in one animal sacrificed at 12 mo and in 2 animals sacrificed at 18 mo. Four of the lungs examined at 22 mo contained an adenoma, and another lung contained multiple adenomas. Multiple adenomas were found in the lung of 1 of 9 rats that died between 18 and 22 mo.

The major reaction seen in the lungs of the feldspar-treated rats was granulomatous inflammation which was present at all sacrifice periods. Scattered small granulomas (Fig. 5) were present in all lobes of the lung, but in the left lobe the reaction was more severe and was occasionally replaced normal pulmonary structures. Macrophages containing birefringent particles were present in the alveoli at all sacrifice intervals. Scattered areas of alveolar wall fibrosis and type II epithelial hyperplasia were evident at all sacrifices. Chronic bronchitis and bronchiectasis were present in a few lungs, primarily at the 22-mo sacrifice. Chronic tracheitis was evident in five animals sacrificed at 22 mo. A diffuse granulomatous reaction was present in the tracheobronchial lymph nodes at all sacrifice periods. Anisotropic particles were present in these nodes at all sacrifice intervals.

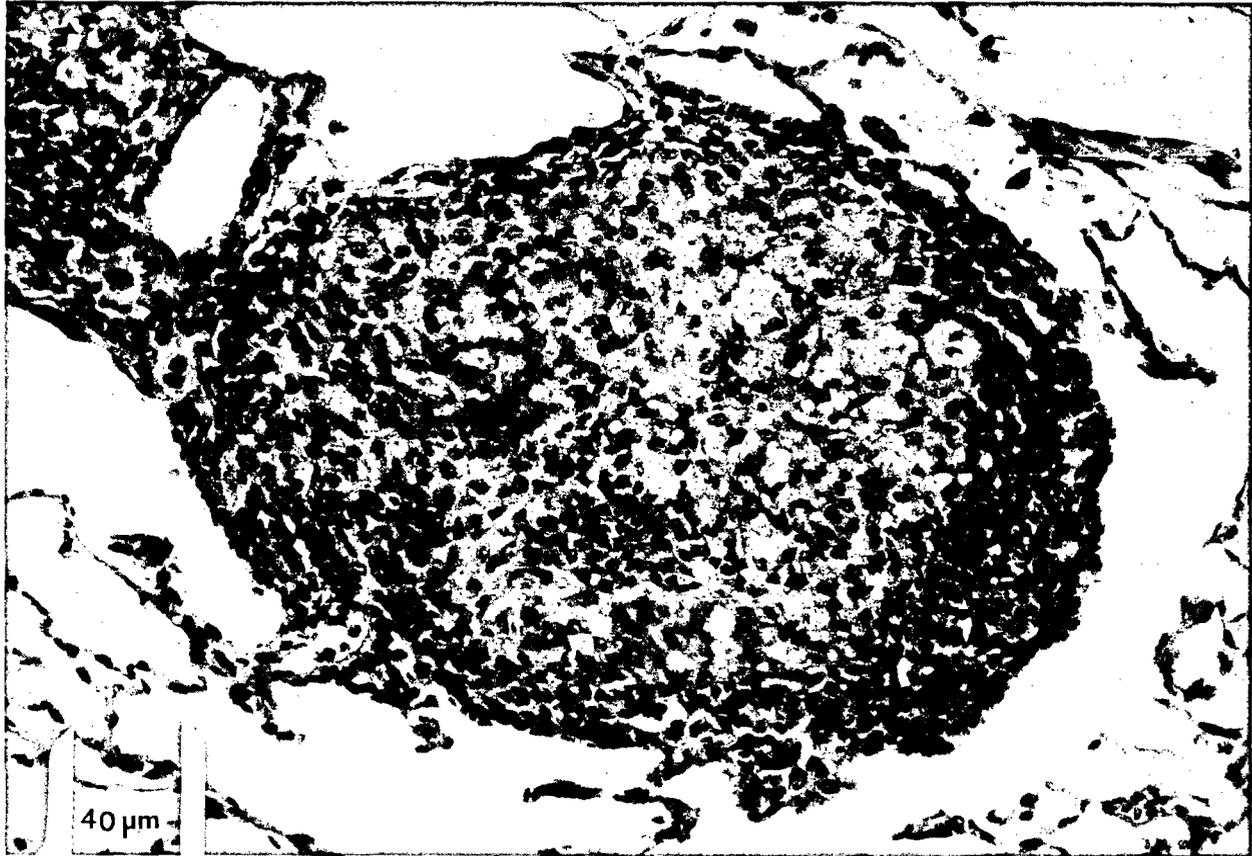


FIGURE 5. Light micrograph of a 5- μ m-thick H&E-stained section of lung from a feldspar-treated rat at 22 mo showing a granuloma. The granuloma is composed of numerous macrophages with a lesser number of lymphocytes concentrated primarily around the margin.

Min-U-Sil

Thirty rats from the Min-U-Sil treatment group were found to have primary lung tumors. The first tumor, an adenoma, was seen in a rat from the 12-mo sacrifice. At 18 mo, multiple adenomas were present in 1 rat while adenocarcinomas were present in 2 other rats. Three of the 12 rats that died between 18 and 22 mo also had primary lung tumors: one had an adenoma and an adenocarcinoma, another animal had multiple adenomas and an adenocarcinoma, and the third animal had only an adenocarcinoma. Of the 30 animals sacrificed at 22 mo, primary lung tumors were present in 23 of these animals. Ten of the 30 lungs contained only an adenoma, 1 contained an adenoma and an adenocarcinoma, 1 lung contained multiple adenomas, 5 lungs contained multiple adenomas and an adenocarcinoma, 5 contained adenocarcinomas, and 1 lung contained multiple adenomas and a keratinizing epithelioma.

The pulmonary reaction seen in the Min-U-Sil-treated rats was the most extensive seen in any of the treatment groups. Chronic granulomatous inflammation was present in the lungs at all sacrifice periods. Scattered small granulomas were present in all lobes of the lungs; however, the most severe lesions were present in the left lobe, where a diffuse granulomatous reaction was evident which obliterated much of the pulmonary parenchyma. Fibrosis, ranging from slight to severe, was evident in the granulomatous reaction, primarily at the 22-mo sacrifice period (Fig. 6). Multifocal alveolar macrophages were present at all sacrifice intervals. Some of these macrophages were necrotic, beginning with the 12-mo sacrifice. There was an associated type II epithelial hyperplasia, ranging from minimal to moderately severe, at all sacrifice periods, and/or there was minimal to moderate alveolar wall fibrosis, particularly at the 18- and 22-mo sacrifices. Chronic bronchitis, ranging from moderate to moderately severe, was present in many of the lungs, particularly those at 22 mo (15/30). As with the control rats, peribronchial mononuclear infiltrates were present in the lungs of the Min-U-Sil-treated rats, but unlike the controls, these lungs contained small granulomas. Chronic pleuritis, ranging from slight to moderately severe, was present in most of the animals from the 12-, 18-, and 22-mo sacrifices. Particulate material and diffuse granulomatous inflammation were noted in the tracheobronchial lymph nodes at all sacrifice intervals. This inflammation was responsible for the gross enlargement of the nodes. Chronic tracheitis was evident in 3 of 30 animals at the 22-mo sacrifice.

Novaculite

Twenty animals from the novaculite test group were found to have primary lung tumors. One of 10 animals sacrificed at 12 mo contained a

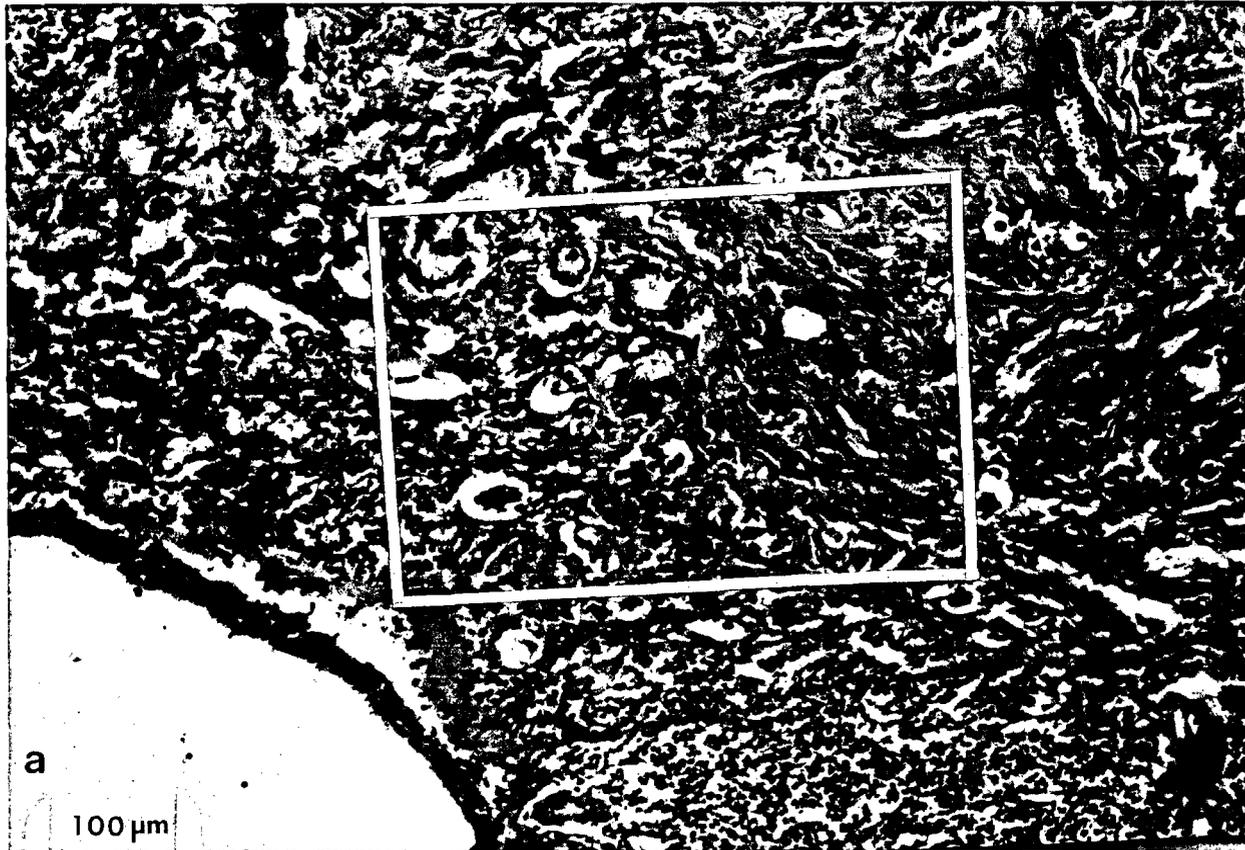


FIGURE 6. (a) Light micrograph of a 5- μm -thick H&E-stained section of lung from a Min-U-Sil-treated rat at 22 mo. Inflammation and fibrosis has obliterated most of the pulmonary parenchyma.

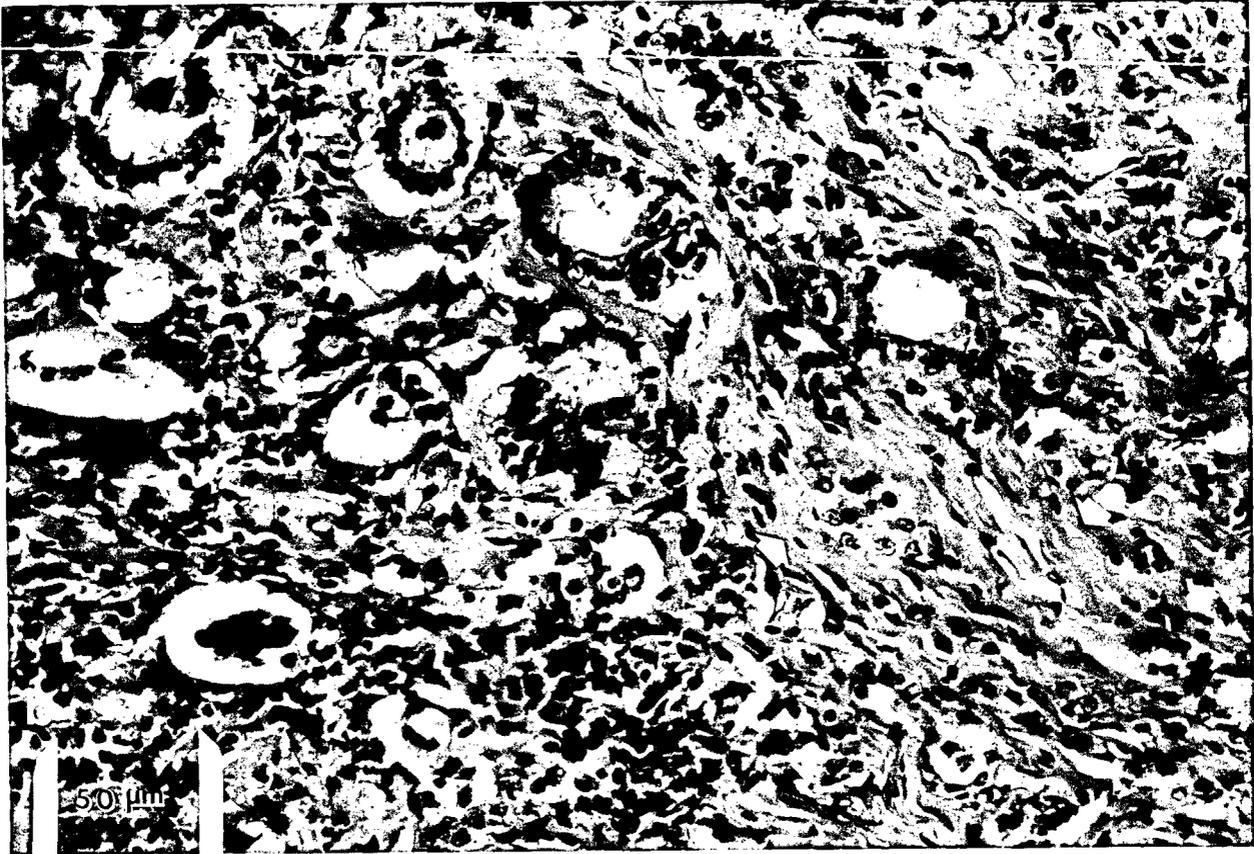


FIGURE 6. (Continued) (b) Enlargement of the inset in (a). There is an abundant amount of variably mature fibrous connective tissue (arrows) admixed with numerous mononuclear inflammatory cells.

pulmonary adenoma. Two of the 18-mo sacrifice animals contained pulmonary neoplasms. One had an adenoma and another had both an adenoma and an adenocarcinoma. Two of 11 animals that died between 18 and 22 mo had an adenoma in the lung, and a third rat had a pulmonary squamous-cell carcinoma. Fourteen of 34 animals sacrificed at 22 mo had lung tumors; 8 rats had an adenoma, 4 had multiple adenomas, and 2 had adenocarcinomas.

Nonneoplastic changes produced in novaculite-treated rats were qualitatively similar to those produced by Min-U-Sil. Multiple small granulomas were scattered throughout the right lung of most animals. A multifocal to diffuse granulomatous reaction was present in the left lung lobe of most animals. In some areas this granulomatous inflammation obscured normal alveolar structures much like that seen with Min-U-Sil; however, small spherical granulomas were evident throughout this diffuse reaction. In general, the granulomatous reaction seen with novaculite was slightly less severe than the Min-U-Sil-treated rats. In contrast to the Min-U-Sil-treated rats, fibrosis ranging from minimal to moderately severe was seen in only a few of the novaculite-treated rats in the granulomatous areas. Alveolar wall fibrosis and type II epithelial hyperplasia in alveoli adjacent to areas of granulomatous inflammation were qualitatively and quantitatively similar to that seen with Min-U-Sil. Microgranulomas were also evident in the peribronchial mononuclear infiltrates. Chronic bronchitis was present in some animals, as was a chronic granulomatous pleuritis.

Statistical Analysis of Tumor Data

The expected proportion of rats having primary pulmonary tumors was modeled as a logit-linear function (McCullagh and Nelder, 1983), of treatment group, time of sacrifice or death (months after intratracheal instillation), and a scheduled sacrifice/spontaneous death dichotomy. Models were fitted by maximum likelihood, and significance testes of relevant parameters were performed using the approximating chi-square distribution to the likelihood ratio test statistic. Model fit was assessed by computing the deviance statistic, a grouped Pearson chi-square statistic (Cox, 1970), and by examining likelihood ratio tests of more flexible models with additional parameters.

The model reported in Table 5 has acceptable fit to the data (Pearson chi-square = 24.16, $df = 19$, $p = 0.190$). Other models examined with additional parameters did not yield a significant improvement. This model has a slope parameter multiplying time measured in months (= 30.4 d) for each treatment group and two intercepts, one for rats undergoing scheduled sacrifice and the other for those that died or were sacrificed moribund. The slope and intercept parameters define a linear relationship between $\text{logit}(p) = \log[p/(1 - p)]$ and time, where p is the expected proportion of tumor-bearing rats. The fitted proportions

TABLE 5. Results of Logistic Model for Pulmonary Tumor Prevalence Fitted by Maximum Likelihood Estimation

Parameter	Treatment group	Parameter estimate	Standard error	<i>p</i> -Values of contrasts of groups vs.		
				Controls	Min-U-Sil	Novaculite
Slopes (mo^{-1})	Primary copper slag	0.1622	0.0429	0.005	<0.001	0.002
	Secondary copper slag	0.1488	0.0440	0.022	<0.001	<0.001
	Nickel slag	0.0809	0.0556	0.663	<0.001	<0.001
	Feldspar	0.1603	0.0431	0.007	<0.001	0.002
	Min-U-Sil	0.2734	0.0443	<0.001		
	Novaculite	0.2258	0.0429	<0.001	0.012	
	Vehicle control	0.0553	0.0615			
Intercepts	Scheduled sacrifices	-5.288	0.861			
	Died/sacrificed moribund	-6.215	0.895			
Deviance = 73.53		df = 122				

from this logistic model for each treatment group at each observation time are equal to $1/\{1 + \exp[-\text{intercept} - (\text{slope} \times \text{time})]\}$. Tests of the treatment groups (i.e., model slope parameters) indicated that all groups except nickel slag have significantly elevated pulmonary tumor proportions relative to the vehicle controls ($p \leq 0.022$, Table 5). The Min-U-Sil and novaculite groups have significantly higher tumor proportions relative to the other treatments ($p \leq 0.002$), with the Min-U-Sil being higher than the novaculite ($p = 0.012$). The rats that died or were sacrificed moribund have a significant reduction in the proportion of tumors relative to those undergoing scheduled sacrifice ($p = 0.018$, test of model intercept parameters).

DISCUSSION

The health hazard of human exposure to crystalline silica has been well documented (e.g., Ziskind et al., 1976). Coal slags, which are waste products from the burning of coal in electric power plants, and copper slags are being used as replacements for silica sand in abrasive blasting operations because of their low crystalline silica contents (<1%). These materials were not tested in an animal model prior to their introduction into commerce. Hence, our interest has been to assess the toxicity of these materials through experimental animals studies.

This study was designed to determine the carcinogenic and fibrogenic potentials of three slag substitutes in rats and to compare those results with the results seen in rats treated similarly with quartz. The fibrogenic responses seen in the lungs of rats from all three slag treatment groups were much less than those seen in the silica treatment groups. No dense areas of fibrosis were seen in any of the slag treatment groups. Only minimal to slight alveolar wall fibrosis was seen in the two copper slag treatment groups, and this response decreased with time, indicating a repair of the lesion with clearance of the dusts. The response seen in the nickel slag treatment group was consistent with that of a foreign body reaction with only minimal fibrosis seen in an occasional animal.

In the current study, no granulomas were formed in the rats treated with the three mineral slags whereas granuloma formation was a primary response seen in rats treated with copper slags in an earlier study (Mackay et al., 1980). In the initial study, Sprague-Dawley rats were used and sacrificed 10 mo post exposure, while Fischer 344 rats were used in the current study. It is unknown whether this inconsistency in granuloma formation is due to differences in the chemical composition of the test agents or due to the difference in the strain of rat used.

With the completion of the current study, we have now evaluated in our laboratory the fibrogenic potentials of five mineral slags: one coal slag, three copper slags, and one nickel slag. All five of these test agents

have produced in rats fibrotic changes that are markedly less severe than the reactions seen in rats similarly treated with quartz.

The carcinogenicity data in the current experiments are very interesting. Both copper slags, the feldspar, and the two quartz samples produced significantly elevated lung tumor responses when compared to the vehicle controls, while the nickel slag failed to produce an elevated tumor response. Furthermore, the lung tumor response seen with both copper slags and with the feldspar was significantly less than that seen for both quartz samples tested. On this basis, one may conclude that while both copper slags and feldspar were carcinogenic to the rats used in and under the conditions of this experiment, they were less so than the quartz samples.

If the carcinogenicity of the smelter slags is related to their trace metal chemistry, the fact that the nickel slag was not carcinogenic suggests that neither the chromium nor the nickel components of the slags are very important for carcinogenesis. On the other hand, the carcinogenicity of these materials may still be related to other carcinogenic components, beryllium in the case of the secondary smelter slag and arsenic in the primary slag. While one may point to the beryllium and arsenic contents of the slags as a possible reason for their carcinogenicity, there is no such obvious reason for the feldspar and quartz samples. The trace element contents of these materials show little in the way of suspect carcinogens.

The carcinogenicity data for the quartz samples used in this study, Min-U-Sil and novaculite, have been reported before (Groth et al., 1986). In the current report, the histopathological slides have been read by two pathologists (J. E. Proctor and R. J. Carolan), and the results of tumor incidence agreed quite well with those of the first pathologist to read the slides (D. H. Groth). The ability of quartz to induce lung tumors in rats by inhalation and intratracheal instillation has been noted in several recent studies, in addition to this study (Holland et al., 1983, 1986; Dagle et al., 1986). To the best of our knowledge, quartz has been found to induce lung tumors only in rats. Recent studies (Renne et al., 1985; Niemeier et al., 1986) using Min-U-Sil from the same lot used in our study failed to produce lung tumors in hamsters following multiple intratracheal instillations. Further work is obviously needed on the question of quartz carcinogenicity. Long-term animal studies are needed in multiple species as well as additional epidemiologic studies.

Coal and copper slags have been used in commerce for nearly 20 yr. Further animal experimentation and epidemiologic studies are also needed for these materials. The three slags tested in this study were chosen because they contained high levels of suspect carcinogens. On the basis of the data presented in this report, one must conclude that both copper slags were carcinogenic to rats.

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Received September 11, 1987

Accepted January 28, 1988