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FEDERAL EXPRESS

TSCA 8(e) Submission

"Histopathologic Observations From a Silicon Carbide Fiber Inhalation Study in Rats"

This notice is being submitted by [REDACTED] for your information, pursuant to the Toxic Substances Control Act (TSCA) Section 8(e). [REDACTED] is asserting a business confidentiality claim covering all of the information contained in this notice.

The [REDACTED] of the [REDACTED] conducted an inhalation study on the biological effects of silicon carbide fibers in rats. In this study, male Fischer rats were exposed to silicon carbide fibers at a high dose (10 mg/m³) for 90 days, 18 hours per day, seven days per week. The animals were removed at this point from exposure and were maintained under normal room environment for 22 months.

At the end of this 22-month observation period, animal tissues were prepared for histopathologic examination by [REDACTED]. Critical tissue slides (respiratory system) were selected by [REDACTED]'s pathologist and submitted to Dr. [REDACTED], D.V.M., Ph.D. of [REDACTED] for evaluation. Both [REDACTED] summary and Dr. [REDACTED]'s review are included in this submission.

[REDACTED]

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Although [REDACTED] has not completed an internal review of these observations and a final study report has not yet been issued, it was deemed appropriate to inform the EPA of these preliminary results through the TSCA 8(e) mechanism.

The technologies utilizing silicon carbide fibers currently being pursued by [REDACTED] will provide a competitive advantage and, therefore, this research is considered proprietary and confidential. Redacted versions of this cover letter and the study results are being provided in this submittal.

Should you require any additional information, please contact Dr. [REDACTED] at [REDACTED] or the undersigned at [REDACTED]

Sincerely,

[REDACTED]

Attachments

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REDACTED VERSION OF TSCA 8(e)

CONFIDENTIAL BUSINESS INFORMATION

**"HISTOPATHOLOGIC OBSERVATIONS FROM A SILICON
- CARBIDE FIBER INHALATION STUDY IN RATS"**

MAY 2, 1995

CONFIDENTIAL

Date: 4/11/95

Client:

[REDACTED]

Reviewing
Pathologist:

[REDACTED]

Study: Histologic review for the database on silicon carbide whisker health effects.

Histopathologic Summary

I. Introduction and Methods

This report describes the biologically significant neoplastic and nonneoplastic changes in the respiratory tract of rats exposed to silicon carbide fibers (SiC). It also briefly discusses the other lesions observed which were not biologically different between the SiC-exposed rats and air-exposed controls. Summaries of the incidences of the principal lung lesions and the individual animal diagnoses are presented in the appendix.

Tissues from the lung (right apical, right medial, right caudal, and left lateral lobes), trachea, and right bronchial lymph nodes were microscopically examined from rats exposed for 90 days (18 h/day, 7 days/wk) to air (controls) or silicon carbide whiskers. This study contained four experimental groups. There were two control and two silicon carbide-exposed groups. In one of the control and one of the silicon carbide-exposed groups, the rats were tested for pulmonary function. The consulting pathologist, [REDACTED] also reviewed the primary pathology report from the [REDACTED], Preliminary Silicon Carbide Inhalation Study Pathology Report).

Severity scores for the principal nonneoplastic lesions were determined by subjective assessment of the percentages of the tissue structures (e.g. alveolar ducts in lung) that were altered. Lesions were minimal (score = 1) if less than 10% of the specific tissue structures in the lung were altered. A

score of 2 (mild) was given to a lesion that involved 10-24% of the specific pulmonary structures and a score of 3 (moderate) for a lesion involving 25-50% of the specific pulmonary structures. The lesion was marked (score = 4) if it involved more than 50% of the specific structures in the lung tissue. Two non-neoplastic alterations (i.e., uremic pneumonia, autolysis) and all neoplastic lesions (e.g., bronchiolar adenoma, mononuclear cell leukemia) were not given a severity score, but were recorded as "P" if present in the tissue. Terminology of all lesions used in this report is defined in the Glossary of Pathological Terms (see appendix).

II. Histopathology

A. Control Group (No Pulmonary Function Tests; n = 31)

1. Non-neoplastic Lesions

Most of the rats in this group had no histologic evidence of spontaneous disease. Nine of the rats in this group, however, had chronic active bronchopneumonia induced by aspiration of plant material from the bedding or food. The severity of this lesion ranged from mild to severe. This lesion was characterized by aggregations of large, vacuolated macrophages, monocytes, and multinucleated giant cells in the several centriacinar regions of the lung. Small amounts of foreign material could be found in the center of a few of these cellular aggregates in the pneumonic lungs. Varying numbers of neutrophils were also part of this inflammatory response and represent the "active" (on-going) aspect of this lesion. Epithelial hyperplasia (i.e., type two pneumocyte proliferation) often accompanied the inflammatory response in the affected alveolar or alveolar duct regions. In addition, there was often neutrophilic infiltration in the more proximal bronchiolar airways associated with a hyperplastic surface epithelium containing numerous mucous (goblet) cells (mucous cell metaplasia). Tracheal epithelium in these rats often had varying degrees of inflammation and epithelial hyperplasia with increased numbers of mucous cells. Foreign-body(aspiration)-induced bronchopneumonia is a common, spontaneous lung lesion observed in laboratory rats that are part of chronic inhalation studies.

2. Neoplastic Lesions

Another spontaneous alteration observed in a few of the rats was metastatic mononuclear cell leukemia (MCL). Seven out of the 31 rats examined in this group had evidence of intrapulmonary MCL characterized by an infiltration of septal capillaries with numerous neoplastic

mononuclear leukocytes. In several rats the infiltration of these neoplastic cells involved interstitial tissues causing septal thickening and secondary alveolar epithelial cell proliferation. Some of the rats with pulmonary MCL also involved perivascular, peribronchiolar, and pleural compartments of the lung. MCL is one of the most common spontaneous neoplastic diseases in F344 rats over 20 months of age. The incidence of MCL in the present group of controls was similar to that historically described in the literature for this strain of rat (Boorman, 1990).

No primary pulmonary tumors were observed in any of the lung tissues examined from this group of control rats.

B. Control Group (Subjected to Pulmonary Function Tests; n = 17)

1. Non-neoplastic Lesions

As in the previous control group, few rats in this group of controls had evidence of airway lesions. Three rats did have histologic evidence of chronic active bronchopneumonia that ranged in severity from minimal to severe. These lesions were interpreted to be caused by aspiration of foreign body material (i.e., food or bedding material).

2. Neoplastic Lesions

No primary neoplasms were evident in any of the lung sections examined from this group of control rats.

Intrapulmonary evidence of MCL was found in eight rats. As mentioned previously, MCL is a common spontaneous leukemia of aged F344 rats. Neoplastic mononuclear leukocytes often infiltrate multiple organs of leukemic rats (i.e., spleen, lung, liver).

C. SiC-Exposed Group (No Pulmonary Function Tests; n = 36)

1. Non-neoplastic Lesions

In contrast to the lungs from control rats, rats exposed to SiC had conspicuous accumulations of dark brown - black fibers (SiC material). The amount and distribution of these inhaled fibers did not significantly differ among the different lung lobes of an individual animal. In addition, the amount of the intrapulmonary deposited material was similar

among the rats examined. Most of these fiber aggregates were present in the interstitial tissue of alveolar ducts and adjacent alveoli. Lesser amounts of fibers were observed in the pleura, perivascular interstitium, peribronchial interstitium, and bronchial associated lymphoid tissue. Interstitial fibers were either within aggregates of interstitial macrophages or free within the interstitium. Approximately 50-60% of the alveolar ducts contained SiC fibers (moderate severity, grade 3). Some of these affected areas had small accumulations of fiber-containing macrophages (alveolar macrophage hyperplasia) in the alveolar airspaces. Mild alveolar epithelial hyperplasia (type two pneumocyte hyperplasia) was another consistent feature of the fiber-related alterations. Neutrophils were rarely associated with the fiber accumulations in the interstitium or alveolar air spaces

Some of the rats also had other fiber-related lesions including minimal-mild septal fibrosis in the affected alveolar duct/alveolar regions (n = 24), minimal-moderate pleural fibrosis (n = 9), and/or hyperplastic epithelial foci (n = 3). The latter lesion was characterized by conspicuous focal areas of marked epithelial hyperplasia lining mildly fibrotic alveolar septa.

Chronic, active bronchopneumonia was evident in four SiC-exposed rats. This lesion was interpreted to be due to the aspiration of food or bedding material and not related to the SiC exposure.

Bronchial lymph nodes examined from SiC-exposed rats often had conspicuous accumulations of SiC fibers and associated lymphoid hyperplasia.

2. Neoplastic Lesions

Five rats (R283, R410, R465, R468, and R481) in this group of SiC-exposed rats had solitary, bronchiolar/alveolar adenomas. All of these primary, benign tumor masses were in the periphery of the lung (parenchyma). Adenomas had an alveolar growth pattern consisting of neoplastic epithelial cells growing along the alveolar septa or an irregular papillary growth pattern with epithelial cells that were uniform (little pleomorphism), cuboidal or columnar (Fig. 1). Tumor epithelial cells were not anaplastic and consisted of round to oval nuclei with varying amounts of cytoplasm. These benign neoplasms had sparse stroma.

Metastatic MCL was present in the lungs of 12 of these SiC-exposed rats. This neoplastic lesion did not appear to affect the distribution or accumulation of the SiC fibers or the severity and distribution of fiber-related lesions.

D. SiC-Exposed Group (Pulmonary Function Tests; n = 18)

1. Non-neoplastic lesions

The amount and distribution of the SiC fibers in the lungs and lymph nodes of these rats were similar to those of the other SiC-exposed group described above. In addition, the fiber-related alterations in these SiC-exposed lungs and lymph nodes were similar in character, severity, and incidence as those in the other SiC-exposed group.

2. Neoplastic Lesions

Four rats (R307, R437, R462, and R490) in this group of SiC-exposed rats had primary lung tumors. Two of these rats, R307 and R490, both had a bronchiolar/alveolar adenoma and a bronchiolar/alveolar adenocarcinoma. The adenocarcinoma in R307 was metastatic to the diaphragm and regional bronchial lymph node. This malignant neoplasm had a tubular growth pattern characterized by nests or tubules of epithelial cells separated by varying thicknesses of collagenous stroma (Fig. 2). The neoplastic epithelial cells were large, cuboidal or polygonal cells, with a high nuclear to cytoplasmic ratio and orientated around a lumen. A solitary bronchiolar/alveolar adenocarcinoma was also evident in the lung of R462. The bronchiolar/alveolar adenocarcinomas in R462 and R490 had a papillary growth pattern consisting of distinct folds or fronds lined by varying sized columnar or cuboidal epithelial cells. Anaplasia was exhibited in these cells by enlarged size, high nuclear-cytoplasmic ration, and piling-up of individual cells. Invasion of neoplastic cells into adjacent airway walls or peribronchiolar interstium was evident in some of these tumors. As mentioned above, one of these malignant lung tumors metastasized to the adjacent diaphragm.

A solitary, bronchiolar/alveolar adenoma was present in the lung of R437. This benign neoplasm had a papillary growth pattern as did the adenomas in R490 and R307.

Metastatic MCL was present in the lungs of seven rats in this group.

III. Summary

The principal nonneoplastic lesions induced by SiC fiber accumulation were mild alveolar epithelial hyperplasia with mild alveolar macrophage hyperplasia and minimal septal fibrosis. These alterations were associated with accumulations of SiC fibers in the walls of alveolar ducts and associated alveoli. These alterations would be graded a 4 according to the Wagner grading system (cellular change with minimal fibrosis; Wagner, 1974). Interestingly, no active inflammatory response (i.e., neutrophil infiltrate) was associated with these other minor alterations.

There were no significant differences in the amount and distribution of the SiC fibers in the lungs of rats that were subjected to pulmonary function testing and those that were not given pulmonary function tests. In addition,

there were no group-related differences in the severity, character and distribution of the minor nonneoplastic lesions induced by the fiber accumulation.

Primary lung tumors (benign or malignant) were evident only in rats exposed to SiC fibers. Surprisingly, malignant adenocarcinomas of the lung were only found in rats that were exposed to SiC fibers and were subjected to pulmonary function tests. Three of the 18 rats in this group had solitary adenocarcinomas compared to 0 out of 36 rats in the other SiC-exposed group that did not undergo pulmonary function testing. Four of the 18 SiC-exposed rats (22%) that were given pulmonary function tests had some type of primary neoplasm (adenoma or adenocarcinoma). Only 5 of the 36 rats in the other SiC-exposed group (14%) had primary lung tumors (all adenomas). The reason for this difference in tumor response between the two groups of rats is unknown.

The results of this study indicate that repeated inhalation exposures of F344 rats to SiC fibers induce both nonneoplastic and neoplastic pulmonary lesions. In addition, SiC-exposed rats that are subjected to pulmonary function testing may be more susceptible to fiber-induced pulmonary tumors.

IV. [REDACTED] Report

This pathology report is clearly and concisely written. The reviewing pathologist is in agreement with the principal finding of the [REDACTED] pathologist that the SiC fiber exposures increased the incidence of primary lung tumors. There was, however, a difference in interpretation of a proliferative lesion in a rat in the SiC-exposed rat (R283) that was not subjected to pulmonary function tests. The [REDACTED] pathologist reported this lesion to be adenomatous hyperplasia and this reviewing pathologist reported it as a bronchiolar/alveolar adenoma. In addition, the [REDACTED] pathologist reported a bronchiolar/alveolar adenoma in R385 (SiC-exposed group of rats that were given pulmonary function tests) and this reviewing pathologist found no primary lung tumor in this animal.

The reviewing pathologist also did not agree with the conclusion of the [REDACTED] pathologist that no fiber-related nonneoplastic lesions were evident in the SiC-exposed rats. As stated above, there were both mild proliferative and fibrotic lesions associated with the fiber accumulations. The differences in interpretation may be due to the different methods of recording nonneoplastic lesions (e.g., alveolar epithelial hyperplasia, septal fibrosis). For example, the reviewing pathologist separated the nonneoplastic lung lesions that were secondary to the mononuclear cell leukemia (MCL) from the nonneoplastic lesions that were associated with the fiber accumulation. MCL is known to produce secondary proliferative and fibrotic lesions in the lung. These nonneoplastic lesions are often widespread throughout the lung lobe and associated with the neoplastic cell infiltration. In contrast, the nonneoplastic, proliferative and fibrotic lesions associated with SiC fiber

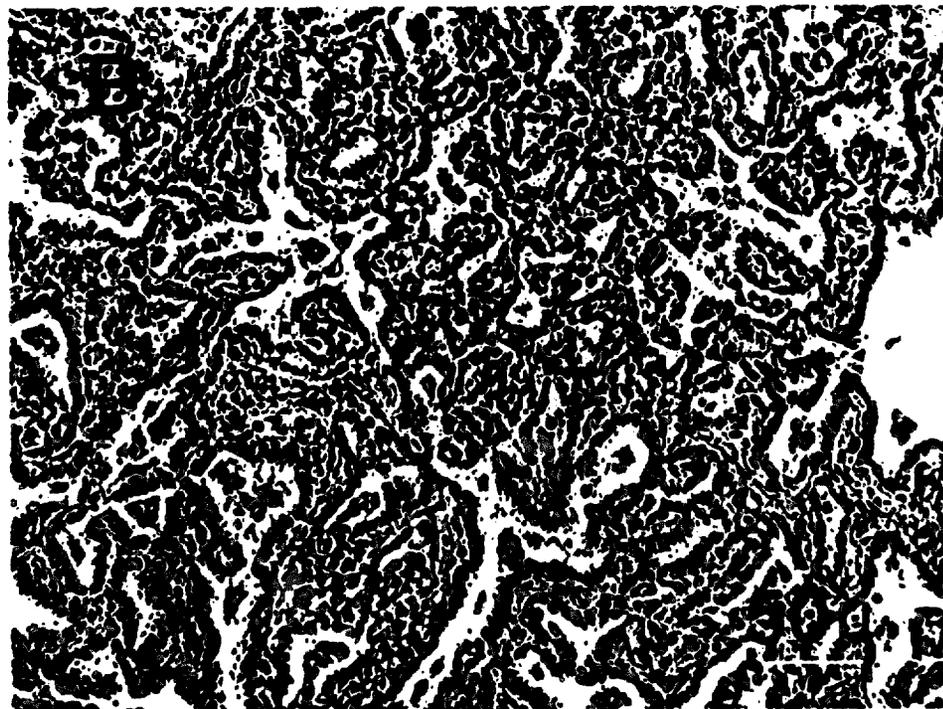
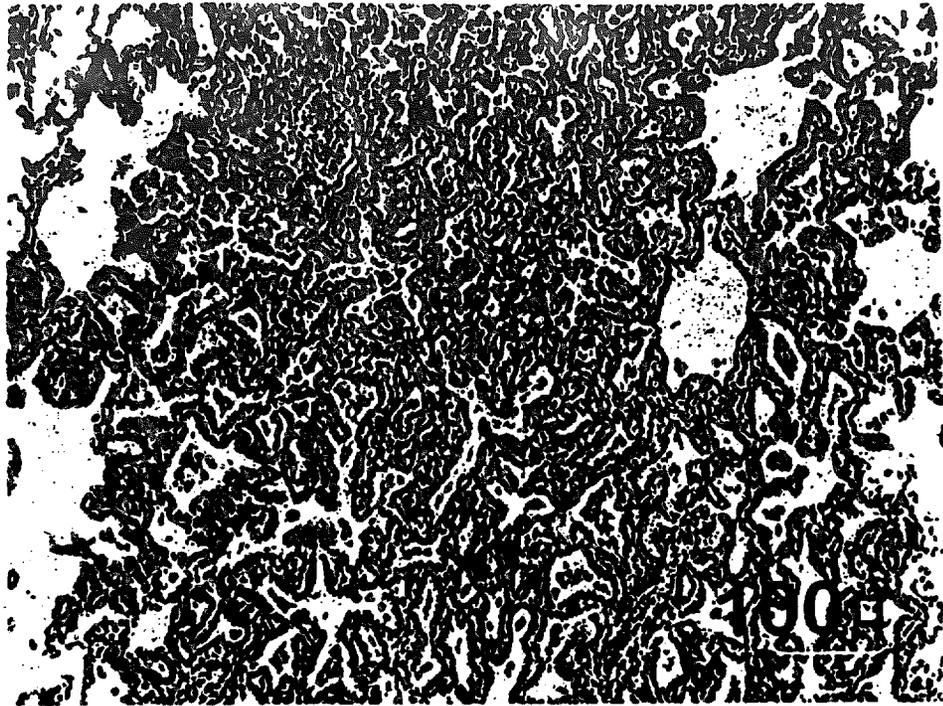
exposure are multifocal and restricted to alveolar duct/alveolar regions that contain large numbers of fibers. Since the [REDACTED] pathologist did not separate the MCL-induced secondary lesions from the fiber-related proliferative lesions, there were no statistically recognizable differences in the proliferative lesions among the control and SiC-exposed groups.

The use of the terms "lymphosarcoma" and "adenomatous hyperplasia" are archaic and inappropriate for this study. They should be replaced by mononuclear cell leukemia and alveolar epithelial hyperplasia (or type two pneumocyte hyperplasia), respectively. The reviewing pathologist found several more rats in all the groups with MCL than the [REDACTED] pathologist. This is, as previously stated, a common spontaneous disease of old F344 rats.

V. References

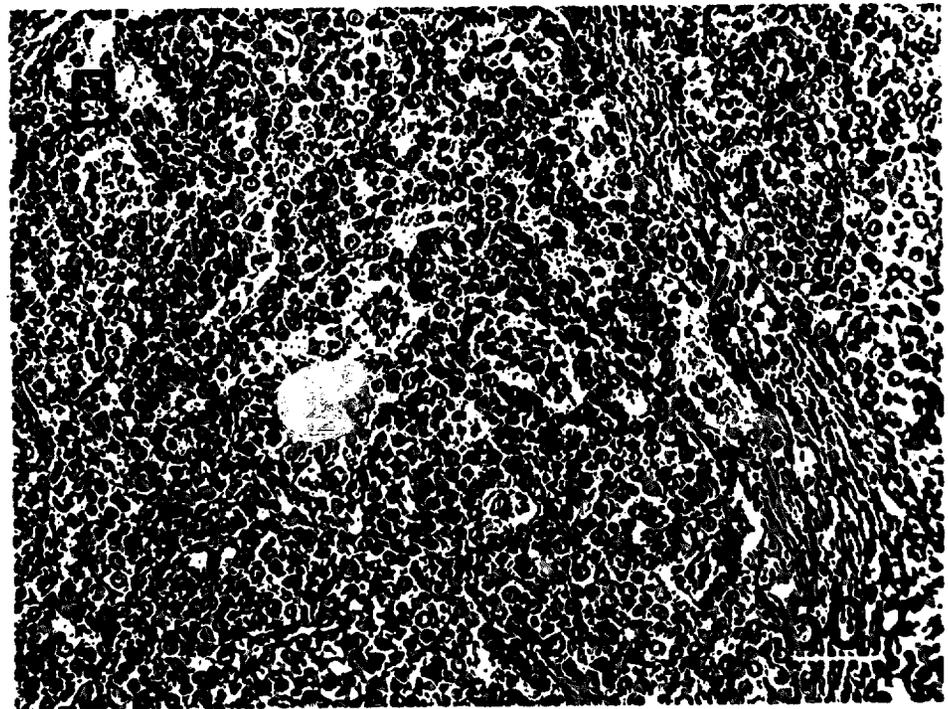
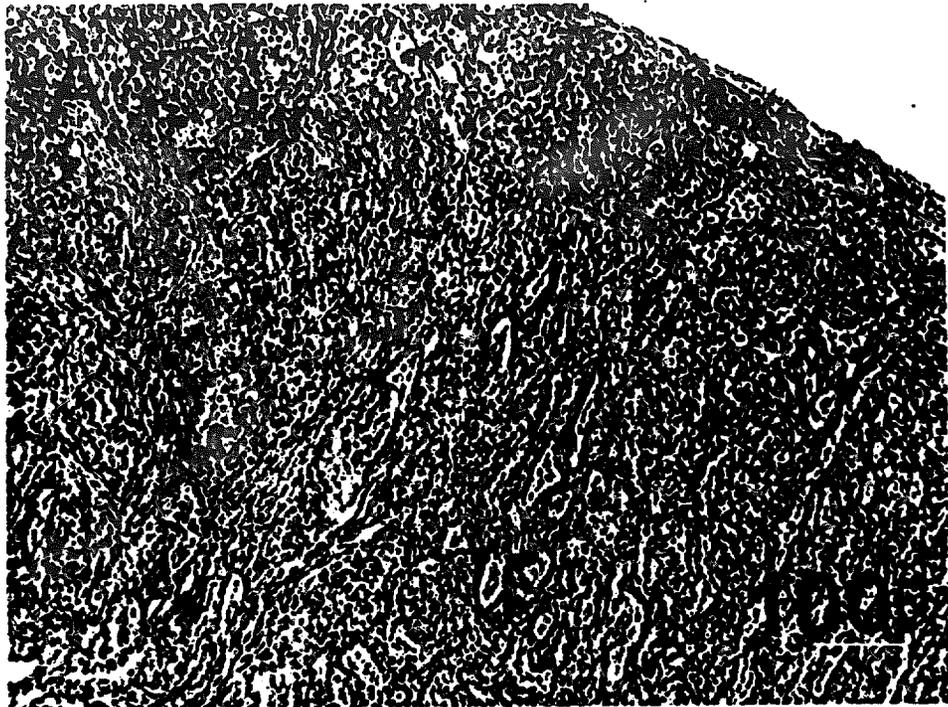
Boorman GA, Eustis SL, Elwell MR, Montgomery CA Jr., and Mackenzie WF (eds) (1990). Pathology of the Fischer Rat, Academic Press, Inc, San Diego.

Wagner JC, et al. (1974). The effects of the inhalation of asbestos in rats. *British Journal of Cancer* 29: 252-269.

FIG. 1

A) Light photomicrograph of a bronchiolar/alveolar adenoma from the lung of R481. This benign neoplasm has a papillary growth pattern.

B) Higher magnification of lung tumor tissue in A.

FIG. 2

A) Light photomicrograph of bronchiolar/alveolar adenocarcinoma from the lung of R307. This malignant neoplasm has a tubular growth pattern.

B) Higher magnification of the lung tumor tissue in A.