

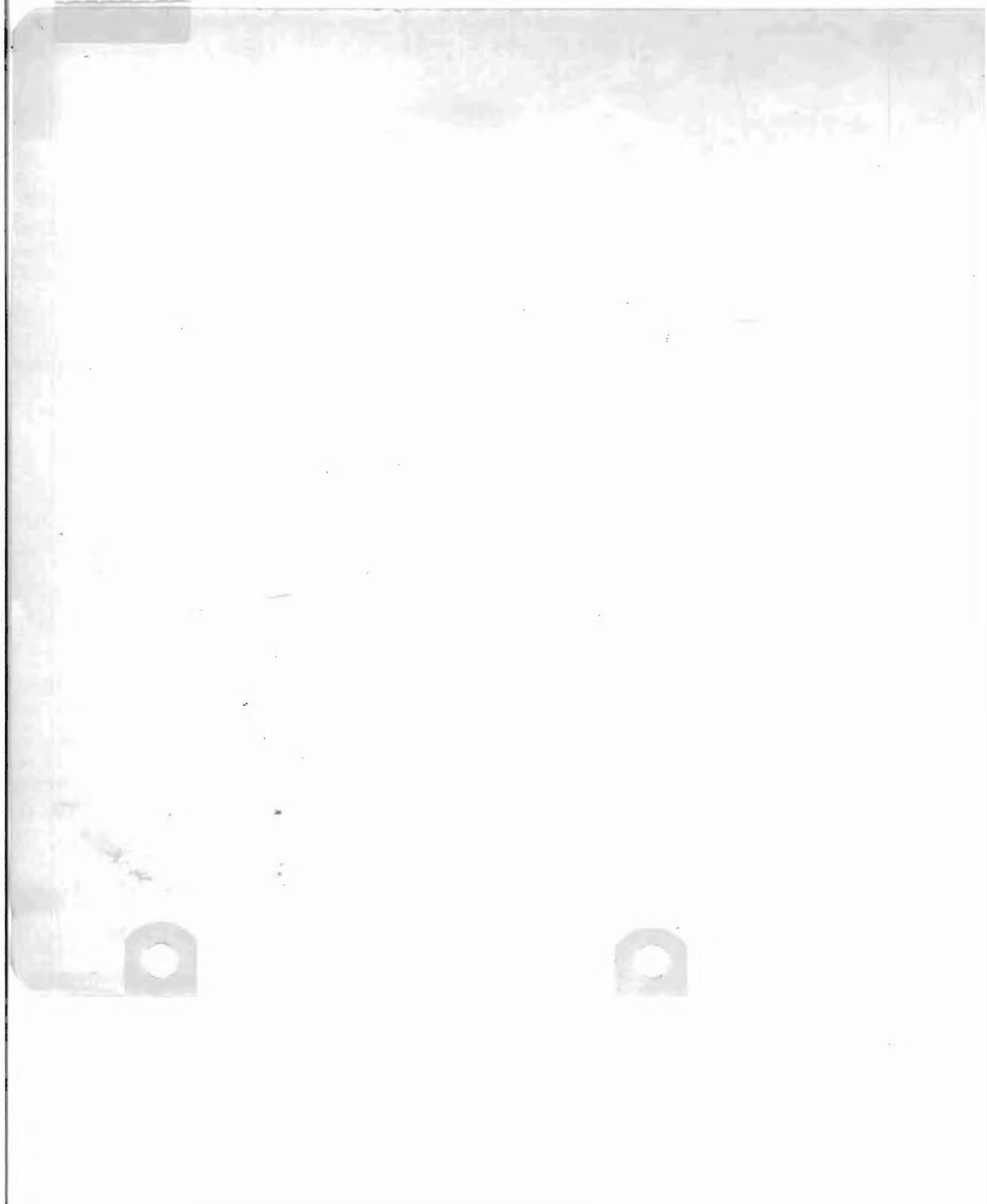
# **REFERENCES**

(FDA Docket No. 94P-0420/CP 1)

## **SECTION II. D**

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## **SECTION IV.**



# An Analysis of the National Toxicology Program's (NTP) Technical Report (NTP TR 421) on the Toxicology and Carcinogenesis Studies of Talc<sup>1</sup>

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The NTP toxicology and carcinogenicity studies of nonasbestiform, cosmetic-grade talc (the NTP Talc Report) were conducted by exposing male and female F344/N rats and B6C3F1 mice to target aerosol concentrations of 0, 6, and 18 mg/m<sup>3</sup> talc for 6 hr daily, 5 days per week. Based on results of the high dose, the Report concluded that talc caused lung tumors in female rats and pheochromocytomas in male and female rats, and there was no evidence of carcinogenic activity in mice. A thorough evaluation of lung toxicity revealed that talc-induced lung tumors occurred only in the group of animals that exhibited the most profound degree of chronic toxicity. However, these data were presented as empirical observations rather than discussed in a manner that would relate them to the risk assessment implications of the bioassay, i.e., relevant data were collected but not "used." In addition, the evaluation of the pheochromocytomas was inadequate because it failed to place sufficient emphasis on the spontaneous incidence of this tumor in rats. These deficiencies caused the author to vote against the conclusions presented in the Talc Report when it was reviewed by the NTP Board of Scientific Counselors. The appropriate conclusions are (1) the data do not indicate that the pheochromocytomas were treatment-related; (2) the maximum tolerated dose (MTD) was exceeded in the female rats exposed to the high dose; and (3) talc is not expected to cause lung tumors under conditions of exposure that fail to result in marked chronic lung toxicity. © 1995 Academic Press, Inc.

## INTRODUCTION

The NTP toxicology and carcinogenicity studies of nonasbestiform, cosmetic-grade talc (the NTP Talc Re-

port) were conducted by exposing male and female F344/N rats and B6C3F1 mice to target aerosol concentrations of 0, 6, and 18 mg/m<sup>3</sup> talc for 6 hr daily, 5 days per week (NTP, 1993). A unique aspect of these studies was the inclusion of a very thorough evaluation of lung toxicity, in addition to the standard gross pathology and histopathology assessments. The NTP Talc Report was reviewed by the NTP Board of Scientific Counselors (The Board), Technical Reports Review Subcommittee on June 23-24, 1992. Jay I. Goodman participated in this review as a member of the Board and a member of the Board's Technical Reports Review Subcommittee. The Board concurred with the recommendations of the NTP staff and voted to approve (by a vote of seven yes votes to one no vote (J. I. Goodman)) the following conclusions: "Under conditions of these inhalation studies there was some evidence of carcinogenic activity of talc in male F344/N rats based on an increased incidence of benign and malignant pheochromocytomas of the adrenal gland. There was clear evidence of carcinogenic activity of talc in female F344/N rats based on increased incidences of alveolar/bronchiolar adenomas and carcinomas of the lung and benign and malignant pheochromocytomas of the adrenal gland. There was no evidence of carcinogenic activity of talc in male or female B6C3F1 mice exposed to 6 or 18 mg/m<sup>3</sup>."

The specific aims of this paper are to focus on the studies involving rats, the species in which there was deemed to be a carcinogenic effect, and (1) to provide an overview of the talc bioassay (NTP, 1993) with an emphasis on the rationale for the author's disagreement with the conclusions reached by the other members of the Board regarding the carcinogenicity of talc and (2) to provide a realistic perspective regarding the significance of the talc bioassay.

## SYNOPSIS OF THE CARCINOGENICITY STUDY OF TALC

Groups of 50 male and 49 or 50 female F344/N rats were exposed to aerosols containing 0, 6, or 18 mg/m<sup>3</sup> talc, 6 hr a day 5 days per week, until mortality in any

<sup>1</sup> Presented, in part, at the International Society of Regulatory Toxicology and Pharmacology/U.S. FDA Workshop on Talc: Consumer Uses and Health Perspectives, NIH, Bethesda, MD, January 31-February 1, 1994.

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**TABLE 1**  
**Summary of the Lifetime and 2-Year Carcinogenicity Studies of Talc<sup>a</sup>**

|                                            | Male F344/N rats                                                               | Female F344/N rats                                                                                                                                       |
|--------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Exposure levels                            | 0, 6, or 18 mg/m <sup>3</sup>                                                  | 0, 6, or 18 mg/m <sup>3</sup>                                                                                                                            |
| Body weights                               | High-dose group slightly lower than controls                                   | High-dose group slightly lower than controls                                                                                                             |
| Survival rates                             | 9/50, 14/50, 16/50                                                             | 11/50, 13/49, 9/50                                                                                                                                       |
| Neoplastic effects                         | Adrenal medulla: benign or malignant pheochromocytoma<br>(26/49, 32/48, 37/47) | Lung: alveolar/bronchiolar adenoma or carcinoma<br>(1/50, 0/48, 13/50)<br>Adrenal medulla: benign or malignant<br>pheochromocytoma (13/48, 14/47, 23/49) |
| Level of evidence of carcinogenic activity | Some evidence                                                                  | Clear evidence                                                                                                                                           |

<sup>a</sup> National Toxicology Program, 1993.

exposure group reached 80% (113 weeks for males and 122 weeks for females). The survival of male and female rats exposed to talc was similar to that of the controls, and the mean body weights of the animals in the high-dose group were only slightly lower (not more than approximately 10% lower) than those of controls after Week 65 (NTP, 1993). The results were interpreted as indicating some evidence of carcinogenicity based on benign and malignant pheochromocytomas in male and female rats and clear evidence of carcinogenicity based on alveolar/bronchiolar adenomas and carcinomas of the lung in female rats. The study is summarized in Table 1.

Groups of 47 to 49 male and 48 to 50 female B6C3F1 mice were exposed to aerosols containing 0, 6, or 18 mg/m<sup>3</sup> talc, 6 hr a day 5 days per week, for up to 103 or 104 weeks. Final mean body weights and survival of the mice exposed to talc were similar to those of the controls (NTP, 1993). The results were interpreted as indicating no evidence of carcinogenic activity in mice.

#### INTERIM EVALUATIONS IN RATS

In a parallel series of ancillary studies, additional groups of animals were exposed to talc, as described above, and examined for interim pathology evaluations or pulmonary function tests after 6, 11, 18, and 24

months and lung biochemistry and cytology studies after 24 months. These are outlined below.

#### PARAMETERS ASSESSED IN THE ANCILLARY STUDIES

- Lung talc burden
- Pulmonary function
- Lung biochemistry; bronchoalveolar lavage fluid was analyzed to determine:
  - Cell injury—lactate dehydrogenase.
  - Chronic inflammation—polymorphonuclear leukocytes, pulmonary macrophages, alkaline phosphatase, and protein.
  - Lysosomal activation— $\beta$ -glucuronidase and acid phosphatase.
  - Response to oxidant injury—increased glutathione reductase.
- Lung collagen metabolism, protein synthesis, and proteinase activity.

#### LUNG TALC BURDEN, LUNG WEIGHTS, AND RESPIRATORY FUNCTION

The lung talc burden at the 6-, 11-, 18-, and 24-month interim sacrifice times was similar in male and

**TABLE 2**  
**Lung Talc Burden (Normalized to Exposure Concentration) of Rats<sup>a</sup>**

|                  | Male                           |                      | Female              |                      |
|------------------|--------------------------------|----------------------|---------------------|----------------------|
|                  | 6 mg/m <sup>3</sup>            | 18 mg/m <sup>3</sup> | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
| 6-Month interim  | 0.439 $\pm$ 0.040 <sup>b</sup> | 0.602 $\pm$ 0.013*   | 0.406 $\pm$ 0.032   | 0.464 $\pm$ 0.007*   |
| 12-Month interim | 0.731 $\pm$ 0.098              | 1.165 $\pm$ 0.113*   | 0.785 $\pm$ 0.043   | 0.787 $\pm$ 0.187    |
| 18-Month interim | 1.22 $\pm$ 0.12                | 1.53 $\pm$ 0.05      | 1.28 $\pm$ 0.06     | 1.35 $\pm$ 0.04      |
| 24-Month interim | 1.74 $\pm$ 0.21                | 1.34 $\pm$ 0.19      | 1.52 $\pm$ 0.15     | 1.63 $\pm$ 0.13      |

<sup>a</sup> Table F3, National Toxicology Program, 1993.

<sup>b</sup> Units are presented as mg talc/g control lung/mg/m<sup>3</sup>.

\* Significantly different ( $P \leq 0.05$ ) from the 6 mg/m<sup>3</sup> group by Dunn's or Shirley's test.

**TABLE 3**  
**Total Lung Capacity of Rats<sup>a</sup>**

|                  | 0 mg/m <sup>3</sup>       | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|------------------|---------------------------|---------------------|----------------------|
| Male             |                           |                     |                      |
| 6-Month interim  | 19.86 ± 0.54 <sup>b</sup> | 19.48 ± 0.46        | 19.25 ± 0.39         |
| 11-Month interim | 20.06 ± 0.32              | 18.44 ± 0.39*       | 17.67 ± 0.45*        |
| 18-Month interim | 20.30 ± 0.45              | 18.87 ± 0.41**      | 16.34 ± 0.52*        |
| 24-Month interim | 20.50 ± 0.83              | 20.20 ± 0.28        | 16.47 ± 1.53         |
| Female           |                           |                     |                      |
| 6-Month interim  | 14.20 ± 0.25              | 14.56 ± 0.27        | 13.80 ± 0.27         |
| 11-Month interim | 13.29 ± 0.21              | 12.91 ± 0.17        | 12.06 ± 0.26*        |
| 18-Month interim | 13.94 ± 0.26              | 12.68 ± 0.28*       | 11.43 ± 0.31*        |
| 24-Month interim | 14.85 ± 0.31              | 13.73 ± 0.34**      | 11.50 ± 1.07*        |

<sup>a</sup> Table F10, National Toxicology Program, 1993.

<sup>b</sup> Units are presented as ml; ratio is (dosed group mean/control group mean) × 100.

\*  $P \leq 0.01$ .

\*\* Significantly different ( $P \leq 0.05$ ) from the control by Dunn's and Shirley's test.

female rats exposed to 6 or 18 mg/m<sup>3</sup> talc, respectively (Table 2).

The absolute and relative lung weights of male rats exposed to 18 mg/m<sup>3</sup> were significantly greater than those of controls at the 6-, 11-, and 18-month interim evaluations and at the end of the lifetime study, while those of female rats exposed to 18 mg/m<sup>3</sup> were significantly greater at the 11-, 18-, and 24-month interim evaluations and at the end of the lifetime study. In both males and females, there was a concentration-related impairment of respiratory function which increased in severity with increasing duration of exposure (NTP, 1993). While decreases in total lung capacity were noted in males and females, statistically significant reductions at the 24-month interim were only seen in females and this occurred at both the 6 and 18 mg/m<sup>3</sup> doses (Table 3).

#### BRONCHOALVEOLAR LAVAGE AND LUNG BIOCHEMISTRY

Following the completion of the pulmonary function tests at the 24-month interim evaluation a thorough assessment of biochemical and cellular parameters that could provide insight regarding lung toxicity was made. Bronchoalveolar lavage was performed on the remaining rats in these groups and the lavage fluid was evaluated for enzymes and protein (Table 4) and cell content (Table 5). In addition, lung collagen metabolism and protein synthesis were assessed (Table 6) and proteinase activity in both lavage fluid and lung homogenate supernatant fluid was evaluated (Table 7, males; and Table 8, females). While dose-related increases in these parameters (indicative of toxicity) were noted in both males and females, there was a larger number of parameters that

showed statistically significant increases in the females and, in general, the magnitude of the increases was higher in the females.

#### ASSESSMENT OF THE NTP TALC REPORT

The NTP Talc Report noted that both *in vitro* and *in vivo* studies indicate that talc is not genotoxic and it included a thorough evaluation of lung toxicity (NTP, 1993). However, these data were presented as empirical observations rather than discussed in a manner that would relate them to the risk assessment implications of the bioassay. In other words, relevant data were collected but not used. In addition, the evaluation of the pheochromocytomas was inadequate because it failed to place sufficient emphasis on the spontaneous incidence of this tumor in rats. These deficiencies caused the author to vote against the conclusions presented in the Talc Report when it was reviewed by the NTP Board of Scientific Counselors. The rationale for this decision in the context of a more realistic perspective on the Report is presented below.

The carcinogen bioassay is a qualitative test; it is not a risk assessment. The use of biological information is required in order to place results of the bioassay into proper perspective and to take a rational approach toward risk assessment (Goodman, 1990, 1994). This notion was endorsed in a recent review of the NTP by the NTP Board of Scientific Counselors where it was stated that "Mechanistic studies should pervade the activities of the NTP. . . . With this emphasis the NTP should be able to strengthen its interpretation of test results in the context of human health. Such interpretation should become a part of reports issued by the NTP" (NTP, 1992; Goodman, 1994). It is appropriate to consider the NTP Talc Report in this context.

Talc-induced lung tumors were not detected in male rats, female mice, or male mice. In rats, the principal toxic lesions associated with inhalation exposure to talc included chronic granulomatous inflammation, alveolar epithelial hyperplasia, squamous metaplasia and squamous cysts, and interstitial fibrosis of the lung. These lesions were accompanied by impaired pulmonary function. While the talc burden in the lungs of males and females was similar (Table 2), the degree of chronic toxicity and inflammation was substantially higher in the females (Tables 3-8; NTP, 1993). In mice, inhalation exposure to talc produced some chronic inflammation. In contrast to rats, alveolar epithelial hyperplasia, squamous metaplasia, and interstitial fibrosis were not observed. Overall, markedly less talc-induced lung toxicity was produced in mice than in rats (NTP, 1993). These observations need to be juxtaposed with the findings regarding lung tumors. It is apparent that an increase in lung tumors was seen only in the test animals that clearly exhibited the highest degree of chronic lung toxicity, the female rats exposed to the high dose (18 mg/m<sup>3</sup>) of talc. The amount of toxicity leads the author to

**TABLE 4**  
**Bronchoalveolar Lavage Fluid Enzymes of Rats at the 24-Month Interim Evaluation<sup>a</sup>**

|                              | 0 mg/m <sup>3</sup> | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|------------------------------|---------------------|---------------------|----------------------|
| <b>Male</b>                  |                     |                     |                      |
| β-Glucuronidase <sup>b</sup> | 1.09 ± 0.40         | 18.86 ± 3.20*       | 89.24 ± 14.24**      |
| Lactate dehydrogenase        | 1634 ± 545          | 3193 ± 606          | 8262 ± 380*          |
| Alkaline phosphatase         | 364.7 ± 147         | 572.8 ± 86.8        | 1604.7 ± 143*        |
| Glutathione reductase        | 103.03 ± 16.43      | 99.35 ± 19.79       | 110.99 ± 0.55*       |
| Total protein <sup>c</sup>   | 1.78 ± 0.40         | 3.12 ± 0.64         | 5.79 ± 0.55*         |
| <b>Female</b>                |                     |                     |                      |
| β-Glucuronidase <sup>b</sup> | 3.33 ± 0.97         | 41.05 ± 4.39**      | 154.16 ± 17.21**     |
| Lactate dehydrogenase        | 1655 ± 266          | 3906 ± 444*         | 14E3 ± 1E3**         |
| Alkaline phosphatase         | 427.8 ± 30.9        | 853.6 ± 79.7**      | 2594.7 ± 221***      |
| Glutathione reductase        | 100.6 ± 1.7         | 135.2 ± 22.4        | 460.0 ± 44.8*        |
| Total protein <sup>c</sup>   | 1.20 ± 0.22         | 4.30 ± 0.36**       | 12.96 ± 0.28**       |

<sup>a</sup> Table F4, National Toxicology Program, 1993.

<sup>b</sup> Units presented as mIU/g control lung.

<sup>c</sup> Units presented as mg/g control lung.

\* Significantly different ( $P \leq 0.05$ ) from the control group by Dunn's and Shirley's test.

\*\*  $P \leq 0.01$ .

conclude that the MTD was exceeded in the female rats exposed to the 18 mg/m<sup>3</sup> dose.

The overall incidences of pheochromocytomas (benign, malignant, or complex) of the adrenal medulla that occurred in the 18 mg/m<sup>3</sup> exposed rats were significantly greater than those of the controls (males,  $P = 0.006$ ; females,  $P = 0.024$ ) (NTP, 1993). However, the spontaneous incidence of these tumors was 53% in males and 27% in females. In the author's view, the criterion for statistical significance should be  $P < 0.01$ . This is consistent with the "guideline" indicating that a compound should be considered carcinogenic if the highest dose produces an increase in a common tumor that is significant at the 1% ( $P < 0.01$ ) level or an increase in a rare tumor that is significant at the 5% ( $P < 0.05$ ) level; otherwise, the compound is regarded as a noncarcinogen (Haseman, 1983; Haseman *et al.*, 1986). A rare neoplasm was de-

fined as a neoplasm occurring with a frequency of less than 1%. Therefore, there is no statistically significant increase in pheochromocytomas in talc-treated female rats and talc should not be deemed carcinogenic at this site in these animals.

There has been a pronounced increase in the spontaneous occurrence of pheochromocytomas in male F344 rats, in studies conducted by the NTP, over the past 10 years (Rao *et al.*, 1990; NTP, 1993). In addition, the high rate of pheochromocytomas in the control female rats in the talc bioassay (13/48, 27%) is in sharp contrast to reports indicating that the spontaneous incidence in control female F344 rats, in NTP studies, has been less than 10% (Rao *et al.*, 1990), e.g., in the range of 5.2–7.1% between 1980 and 1983 (Haseman and Rao, 1992). Unfortunately, the NTP failed to make a comparison of the spontaneous pheochromocytomas observed in the talc

**TABLE 5**  
**Bronchoalveolar Lavage Fluid Cell Populations of Rats at the 24-Month Interim Evaluation<sup>a</sup>**

|                                      | 0 mg/m <sup>3</sup> | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|--------------------------------------|---------------------|---------------------|----------------------|
| <b>Male</b>                          |                     |                     |                      |
| Polymorphonuclear cells <sup>b</sup> | 0.333 ± 0.167       | 24.417 ± 2.557*     | 32.50 ± 3.000*       |
| Lymphocytes                          | 0.000 ± 0.000       | 0.500 ± 0.258       | 0.500 ± 0.500        |
| Macrophages                          | 93.67 ± 3.72        | 70.25 ± 2.53*       | 62.75 ± 1.75*        |
| Epithelial cells                     | 6.00 ± 3.61         | 4.83 ± 1.41         | 4.25 ± 1.75          |
| <b>Female</b>                        |                     |                     |                      |
| Polymorphonuclear cells              | 0.625 ± 0.315       | 25.778 ± 2.673**    | 37.000 ± 1.528**     |
| Lymphocytes                          | 0.000 ± 0.000       | 0.722 ± 0.188*      | 1.333 ± 0.667*       |
| Macrophages                          | 91.38 ± 1.75        | 71.22 ± 2.95**      | 57.33 ± 4.67**       |
| Epithelial cells                     | 8.00 ± 2.01         | 2.28 ± 0.50*        | 4.33 ± 2.60          |

<sup>a</sup> Table F5, National Toxicology Program, 1993.

<sup>b</sup> Units presented as percentage of total cells.

\* Significantly different ( $P \leq 0.05$ ) from the control group by Dunn's and Shirley's test.

\*\*  $P \leq 0.01$ .

**TABLE 6**  
**Lung Collagen Metabolism and Protein Synthesis in Rats at the 24-Month Interim Evaluation<sup>a</sup>**

|                                                | 0 mg/m <sup>3</sup> | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|------------------------------------------------|---------------------|---------------------|----------------------|
| <b>Male</b>                                    |                     |                     |                      |
| Lavage fluid collagenous peptides <sup>b</sup> | 39.79 ± 5.07        | 46.99 ± 6.51        | 79.21 ± 13.73        |
| Total lung collagen <sup>c</sup>               | 13.87 ± 0.60        | 15.98 ± 0.39*       | 18.88 ± 3.35*        |
| Collagen production <sup>d</sup>               | 1.58 ± 0.17         | 1.60 ± 0.17         | 1.63 ± 0.22          |
| Collagen degradation <sup>e</sup>              | 31.67 ± 1.72        | 27.74 ± 1.42        | 9.18 ± 2.38*         |
| Noncollagenous protein synthesis <sup>f</sup>  | 142.1 ± 14.5        | 199.8 ± 22.1*       | 312.2 ± 10.6**       |
| <b>Female</b>                                  |                     |                     |                      |
| Lavage fluid collagenous peptides              | 78.27 ± 11.64       | 115.36 ± 8.61*      | 174.71 ± 13.56**     |
| Total lung collagen                            | 14.32 ± 0.66        | 19.95 ± 1.58*       | 36.47 ± 3.39**       |
| Collagen production                            | 0.982 ± 0.185       | 1.804 ± 0.144*      | 2.264 ± 0.347**      |
| Collagen degradation                           | 14.41 ± 2.44        | 21.59 ± 4.99        | 9.38 ± 1.63          |
| Noncollagenous protein synthesis               | 173.9 ± 34.5        | 325.8 ± 90.9        | 554.3 ± 107*         |

<sup>a</sup> Table F7, National Toxicology Program, 1993.

<sup>b</sup> Units are presented as µg/g control lung.

<sup>c</sup> Units are presented as mg/g control lung.

<sup>d</sup> Units are presented as percentage new protein.

<sup>e</sup> Units are presented as percentage new collagen.

<sup>f</sup> Units are presented as dpm × 10<sup>3</sup>/g control lung.

\* Significantly different ( $P \leq 0.05$ ) from the control group by Dunn's and Shirley's test.

\*\*  $P \leq 0.01$ .

bioassay with the historical spontaneous incidence of pheochromocytomas in rats. This would have been particularly appropriate (as the author indicated during the Board's review of the NTP Talc Report, June 23-24, 1992).

The marked increase in the spontaneous incidence of pheochromocytomas in female rats coupled with the lack of a statistically significant increase of this tumor in the treated animals (when the appropriate  $P$  value is employed) and the trend toward an increasing spontaneous incidence in males indicate that the data pre-

sented in the NTP Talc Report do not warrant a conclusion that talc causes pheochromocytomas in rats.

#### CONCLUSIONS

The appropriate conclusion to be drawn from the NTP Talc Report is that the MTD was exceeded in the female rats exposed to the high dose and talc is not expected to cause lung tumors under conditions of exposure that fail to result in marked chronic lung toxicity. Clear evidence of carcinogenic activity of talc was

**TABLE 7**  
**Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Male Rats at the 24-Month Interim Evaluation<sup>a</sup>**

|                                     | 0 mg/m <sup>3</sup>        | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|-------------------------------------|----------------------------|---------------------|----------------------|
| <b>Lavage fluid</b>                 |                            |                     |                      |
| Acid proteinase                     | 0.994 ± 0.329 <sup>b</sup> | 1.866 ± 0.174       | 4.307 ± 0.218*       |
| Cathepsin D                         | 0.147 ± 0.147              | 0.599 ± 0.150       | 2.420 ± 0.147**      |
| Cathepsin B                         | 0.924 ± 0.415              | 1.267 ± 0.094       | 1.887 ± 0.365        |
| <b>Homogenate supernatant fluid</b> |                            |                     |                      |
| Acid proteinase                     | 10.92 ± 0.64               | 17.51 ± 0.90*       | 25.13 ± 1.50**       |
| Cathepsin D                         | 8.53 ± 0.91                | 14.04 ± 0.62*       | 21.03 ± 1.56**       |
| Cathepsin B                         | 2.39 ± 0.41                | 3.48 ± 0.37         | 4.10 ± 0.06*         |
| Neutral proteinase                  | 0.715 ± 0.168              | 2.417 ± 0.304*      | 4.505 <sup>c</sup>   |
| PMN elastase cathepsin G            | 0.490 ± 0.218              | 1.936 ± 0.242*      | 4.457 ± 0.377**      |
| Macrophage elastase collagenase     | 0.225 ± 0.099              | 0.482 ± 0.077       | 0.000 <sup>c</sup>   |

<sup>a</sup> Table F8, National Toxicology Program, 1993.

<sup>b</sup> Units are presented as mg/hr/mg control lung.

<sup>c</sup>  $n = 1$ ; no statistic calculated.

\* Significantly different ( $P \leq 0.05$ ) from the control group by Dunn's and Shirley's test.

\*\*  $P \leq 0.01$ .

**TABLE 8**  
**Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Female Rats**  
**at the 24-Month Interim Evaluation<sup>a</sup>**

|                                 | 0 mg/m <sup>3</sup>      | 6 mg/m <sup>3</sup> | 18 mg/m <sup>3</sup> |
|---------------------------------|--------------------------|---------------------|----------------------|
| Lavage fluid                    |                          |                     |                      |
| Acid proteinase                 | 1.52 ± 0.12 <sup>b</sup> | 3.46 ± 0.33*        | 6.05 ± 0.73**        |
| Cathepsin D                     | 0.015 ± 0.015            | 1.310 ± 0.292*      | 4.043 ± 0.578**      |
| Cathepsin B                     | 1.61 ± 0.26              | 2.15 ± 0.22         | 2.01 ± 0.17          |
| Homogenate supernatant fluid    |                          |                     |                      |
| Acid proteinase                 | 14.04 ± 0.95             | 29.43 ± 1.18**      | 38.61 ± 1.81**       |
| Cathepsin D                     | 10.05 ± 0.68             | 22.97 ± 1.07**      | 30.25 ± 1.60**       |
| Cathepsin B                     | 3.99 ± 0.58              | 6.46 ± 0.60*        | 8.37 ± 0.42**        |
| Neutral proteinase              | 0.648 ± 0.087            | 5.040 ± 0.418**     | 12.293 ± 1.598**     |
| PMN elastase cathepsin G        | 0.785 ± 0.142            | 4.351 ± 0.261**     | 10.313 ± 2.694**     |
| Macrophage elastase collagenase | 0.054 ± 0.037            | 0.068 ± 0.175*      | 2.012 ± 1.126*       |

<sup>a</sup> Table F8, National Toxicology Program, 1993.

<sup>b</sup> Units are presented as mg/hr/mg control lung.

\* Significantly different ( $P \leq 0.05$ ) from the control group by Dunn's and Shirley's test.

\*\*  $P \leq 0.01$ .

seen only in female rats exposed to the high dose of talc and only under circumstances in which there was evidence of marked chronic lung toxicity. These remarks are not intended to connote that the talc bioassay is invalid. The good news is that under the conditions of this study mice did not develop tumors, male rats did not develop lung tumors, and female rats exposed to the 6 mg/m<sup>3</sup> dose did not develop lung tumors. Furthermore, it is doubtful that the pheochromocytomas observed in the talc-treated rats were treatment related. Thus, at the high dose, talc exhibited equivocal evidence of carcinogenic activity toward the adrenal medulla of male rats.

There is a considerable debate regarding the proper criteria for a MTD (Kociba, 1987). However, in a practical sense the chief concern with the MTD is how the resulting data are used in the risk assessment process (McConnell, 1989). Any high dose, no matter how high, that permits animals to live long enough to develop tumors is not necessarily an appropriate high dose. The recent review of the NTP addressed this issue and concluded that "the implicit assumptions underlying extrapolation from the MTD . . . do not appear to be valid. Therefore, both the criteria for selection of the high dose used and the default criteria that are employed for extrapolation from high dose to low dose must be reevaluated in a critical manner" (NTP, 1992; Goodman, 1994). A consideration of the data presented in the NTP Talc Report leads the author to conclude that it would not be appropriate to use the lung tumor end point in female rats as the basis for a risk assessment involving a linear extrapolation to estimate a theoretical risk to humans that might be exposed to relatively low doses of talc.

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