# In-vivo experimental model for silicosis : fisher 344, sprague dawley and wistar rat strains

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# IN-VIVO EXPERIMENTAL MODEL FOR SILICOSIS: FISHER 344, SPRAGUE DAWLEY AND WISTAR RAT STRAINS.

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## ABSTRACT

The selection of an experimental model for silicosis requires a thorough understanding of a number of different pulmonary parameters specific to the animal that is to be used (e.g., clearance time, penetration curves, anatomical differences...), and of the sensitivity of the strains that are to be used.

The pulmonary response of three rat strains (i.e., Fisher 344, Sprague Dawley, Wistar) to silica dust were compared using two different exposure methods: intra-tracheal injections and inhalation. The test periods lasted 3 months for injection and 6 and 12 months for inhalation.

The histological study of the lung revealed a distinct nodular reaction among Sprague Dawley and Wistar strains. Intra-tracheal injections led to the development of fibrotic nodules among Wistar rats, whereas such silicotic nodules were infrequent among injected Fisher 344 rats, and almost absent when exposure was by inhalation.

Sprague Dawley and Fisher 344 rats showed frequent thickening and metaplasia of the alveolar walls near the terminal bronchioles. This tendency was particularly pronounced among rats exposed by inhalation (especially among Fisher 344 rats).

Evaluation of wet lung weight (i.e., hydroxyproline, lipid and silica lung contents) reveals an increase in the different parameters for Sprague Dawley rats relative to the other two strains, regardless of the type of exposure. It has thus been concluded that Wistar rats are the best experimental model for silicosis, as their pulmonary reaction is more characteristic than that of the other two strains.

## INTRODUCTION

The inhalation of dust containing crystalline silica is known to cause silicosis, a serious lung disease. This disease has been studied extensively over the years [15]; many of these were in-vivo experimental studies on the rat. A major question for in-vivo toxicology studies has, however, never been dealt with: the selection of an experimental model. This selection requires a thorough understanding of a number of different pulmonary parameters specific to the animal that is to be used (e.g., clearance time, penetration curves, anatomical differences...[5, 8, 12]) and of the sensitivity of the strains that are to be used. As with different human populations presenting different levels of sensitivity to pathogenic or toxic substances [6, 11], it is known that different rat strains will vary in their sensitivity to an identical aggression [14]. Numerous studies are to be found in the literature, sometimes using mice, but most often rats of different strains, usually Fisher 344 [1, 2, 3, 4], Wistar and CD (SD) BR [13]. This perhaps explains some of the discrepancies between the different results obtained [2, 3]. In our laboratory, the experiments were carried out with Wistar rats supplied by Iffa Credo (France). A study was started to compare the pulmonary response of three rat strains (i.e., Fisher 344, Sprague Dawley and Wistar) to silica dust using two different exposure methods: intra-tracheal instillation and inhalation.

## MATERIALS AND METHODS

Three different strains of EOPS rats were exposed to α-quartz. The Sprague Dawley (SD) and Wistar (W) strains were supplied by Iffa Credo (France), and the Fisher 344 (F) were supplied by Charles River (France).

Two methods of exposure were used: intra-tracheal instillation and inhalation.

### Intratracheal Instillation

The silica (α-quartz) used was 227 Quartex. Cyclone treatment was used to obtain granulometry corresponding to the alveolar fraction.

| Numerical distribution | |
|---|---|
| 94.29% | <1μ |
| 5.27% | 1 - 2μ |
| 0.44% | 2 - 7μ. |

Prior to instillation, the particles were suspended in saline and then ultrasonically agitated. 10 males and 10 females from each strain received an instillation of 30mg of silica; the control group consisted of 5 males and 5 females from each strains and received saline alone . The animals were housed in a monitored area for a period of 3 months.

**Inhalation**

The silica used was Ni-silica supplied by Moulin des Prés (Offronville, France); this is a mixture of sand and stone ground in a humid environment. Granulometry was carried out using an optical microscope.

| Numerical distribution | |
|---|---|
| 89.12% | <1μ |
| 9.41% | 1 - 2μ |
| 1.32% | 1 - 3.5μ |
| 0.15% | 3.5 - 5μ |

The exposure was of the "Full body" type.

14 males and 14 females from each strain were exposed to a concentration of 100 mg/m$^3$, 6 hours per day, 5 days per week and over 4 weeks. The control group consisted of 6 males and 6 females from each strain. They were not exposed and were housed in identical conditions for the duration of the study. For each strain, 7 exposed males and 7 exposed females, as well as 3 control males and 3 control females, were sacrificed at 6 months, the other animals will be sacrificed at 1 year.

**Histological method**

The animals are sacrificed following standard laboratory procedures at 3 month for the rats from the intra-tracheal instillation group and at 6 and 12 months for the rats exposed by inhalation. The lungs are then removed and weighed. The left lung is preserved after insufflation. Following the histological evaluation, the lung samples are dyed using Hematein-Eosin-Saffron, Sirius red and reticulin.

**Biochemical method**

After having taken the sample of the left lung for histological evaluation, the right lung is once again weighed and, in some cases stored at -20°C until biochemical evaluation. The samples are dried at 137°C overnight. The lipids are extracted in 3 successive stages using ethyl alcohol; they are then quantified after evaporation by weighing. The hydroxyproline is evaluated using Stegemann's method [16], oxidation by chloramine T at pH 6 followed by reaction with paradi-methylaminobenzaldehyde in the presence of chloric acid. The absorbance of the obtained pyrrole derivative is measured at 560 nm. The silica was quantified using the method developed by King [7].

## RESULTS

**Histological results**

At 3 months after intra-tracheal instillation, silicotic nodules are clearly visible among W rats the nodules are numerous, with a slightly greater number among the females. Both males and females often present collagen cores surrounded by a cellular crown. Among SD rats, the nodules are smaller and fewer in number, consisting of cells interspersed with collagen bundles, but quasi a-cellular collagen core is not observed as it is with W rats.

Among F rats, numerous coniotic collections (i.e., areas with angular contours and irregular shapes consisting primarily of a cell and dust mixture) and very few silicotic nodules are observed; the nodules can actually be of greater volume than those observed among SD rats but they never present "collagenized" cores such as those seen with W rats.

All three strains present alveolar lipoproteinosis with foam cells. Thickening of the alveolar wall with inflammatory cells and cubic metaplasia of the alveolar cells are also observed for all strains (usually near the terminal bronchioles). These injuries are less pronounced and of more limited extent among W rats than among the other two species.

Six months after exposure by inhalation, the silicotic nodules are numerous and clearly distinguishable for W rats. They do, however, remain cellular and "collagenized" cores, such as those found after intratracheal instillation, are not observed. SD rats also react by forming silicotic nodules smaller than those observed on W rats. F rats, however, present only angular areas of cells resembling coniotic collections.

The difference is even more pronounced at 12 months after exposure; W rats then present large-volume nodules whereas the round silicotic nodules are not always visible among F rats. Other pulmonary modifications (e.g., alveolar lipoproteinosis with foam cells, thickening of alveolar wall (with cells and collagen), cubic metaplasia of the alveolar cells or "bronchiolization") are observed for all three species. At 12 months, however, these frequency of such modifications is greatest among F rats, followed by SD rats.

**Biochemistry**

The results of biochemical quantification are shown in Figures I & II.

**Table I** **Silica Content** (mg / lung)

**Intratracheal Instillation**

| Males | | | Females | | |
|---|---|---|---|---|---|
| F | SD | W | F | SD | W |
| 15.98 | 14.64 | 16.82 | 16.79 | 13.66 | 16.04 |

**Inhalation**

| Males | | | Females | | |
|---|---|---|---|---|---|
| F | SD | W | F | SD | W |
| 6.76 | 8.52 | 10.91 | 4.15 | 6.45 | 7.04 |

## DISCUSSION

The results of biological quantification for each strain should be interpreted according to the values found among control animals. Little difference is observed between the three strains: After intratracheal instillation, the increase in wet lung weight and in hydroxyproline content can be considered roughly equivalent for the three strains. For exposure by inhalation, F rats seem least reactive and SD the most reactive if only biochemical parameters are taken into account.

Quantifying the silica in the lungs after sacrifice may provide a representation of lung clearance. SD rats seem to present the best clearance after intratracheal instillation. When exposure is by inhalation, F rats present the best clearance, followed by SD and W rats.

The histological examination of the lungs reveals two types of injury. Considering the doses at which the animals were exposed, certain overloading probably occurs [9], Recently described injury due to overloading [10] have been identified: cellular and collagenous thickening of the proximal alveolar walls, coniotic collections, cubic metaplasia of the proximal alveolar cells. These injuries have been described primarily for F rats. In this study, they were found for all strains; they are more pronounced among SD and F rats, and are especially pronounced among F rats exposed by inhalation.

The other type of injury that was observed is considered characteristic of silicosis (i.e., silicotic nodules and alveolar proteinosis with foam cells). The silicotic nodules are not very pronounced or are completely absent among F rats; they are not pronounced among SD rats, whereas the nodules are always clearly visible among W rats, which in this study was the only strain that presented "collagenized" cores after interstitial intra-tracheal instillation.

If only biochemical parameters were to be taken into account, it would seem that SD is the most sensitive strain, and thus the best experimental model.

But when these biochemical results are compared with histological examination, it becomes apparent that W rats present the pathology most characteristic of silicosis, SD rats present a mixture of pathologies, some due to overloading and others to silicosis, which explains the higher biochemical quantification values. As for F rats, most of the observed injuries can be attributed to overloading, and they develop few "silicotic" injuries, which is not apparent from biochemical data alone.

It has thus been concluded that, of the three strains studied (Sprague Dawley, Wistar and Fisher) the best model for the experimental study of silicosis is currently the Wistar strain. It should be noted that the data for a strain can "drift" over the years, and the sensitivity of the animals may thus be altered.

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Figure I

# INTRATRACHEAL INSTILLATION

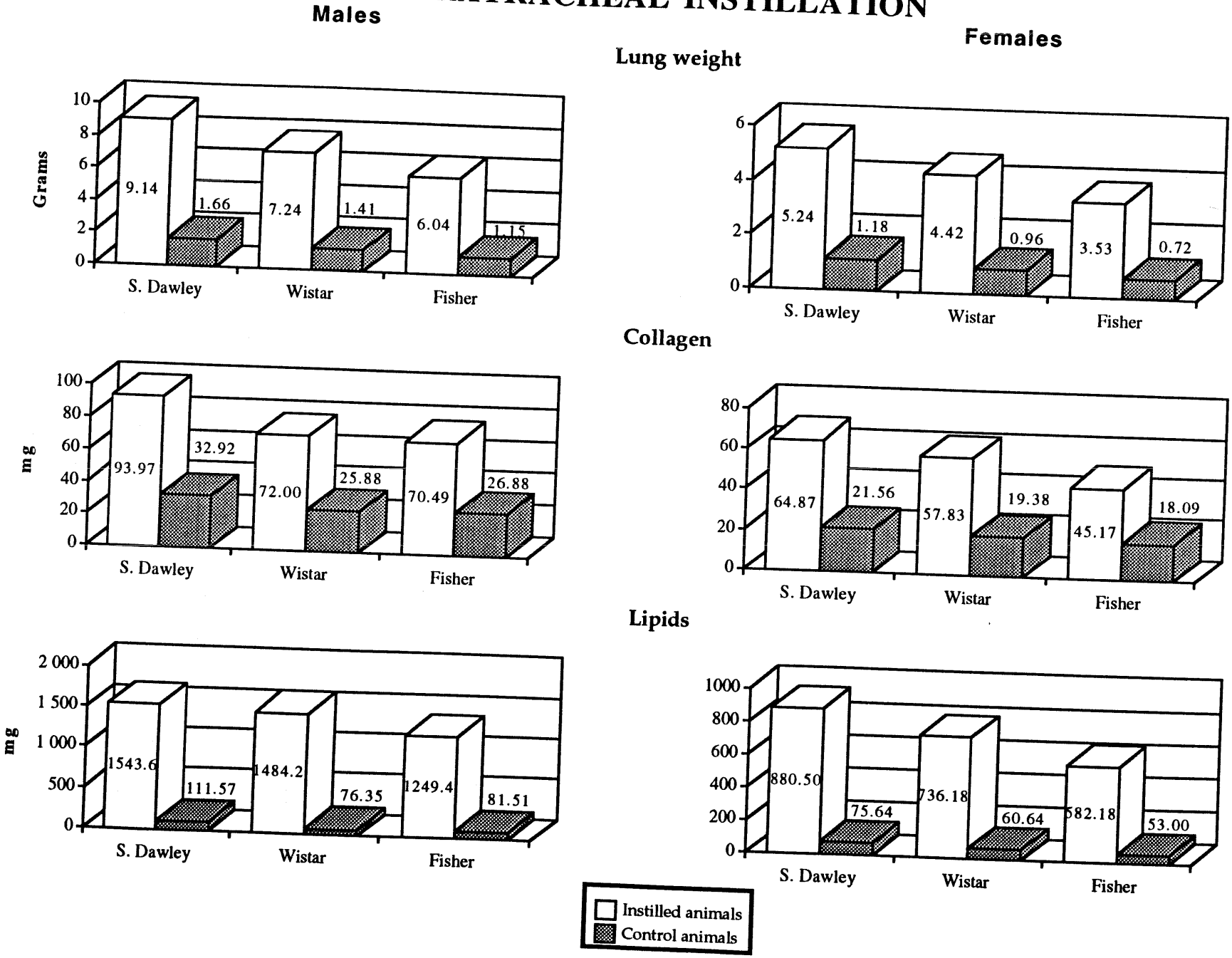

Figure II

# INHALATION (situation at 6 months)