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# Irreversible Pulmonary Changes Induced in Rat Lung by Dust Overload

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**The objective of this study was to investigate whether the effects of dust overload are reversible upon cessation of subchronic exposure to test toner. Female rats were exposed 6 hr/day, 5 days/week for 3 months to a test toner at 0, 10, and 40 mg/m$^3$. The retained quantity of test toner in the lungs at the end of exposure was 0.4 and 3.0 mg for the low and high exposure groups, respectively. Fifteen months later, the corresponding values were 0.12 and 2.65 mg in the lungs. Alveolar clearance of tracer aerosols as well as cytologic and enzymatic parameters in the bronchoalveolar fluid was investigated at the end of exposure and subsequently up to 15 months later. The alveolar clearance of $^{59}Fe_2O_3$, $^{51}$Cr-polystyrene, and $^{85}$Sr-polystyrene tracer aerosols was slightly retarded at the low and substantially impaired at the high exposure level. At the low exposure level, there was some recovery in the clearance behavior up to 6 months after exposure. In contrast, at the high exposure level there was no indication of a reversal of the impaired clearance. For the β-glucuronidase activity and the number of polymorphonuclear cells, the pattern of the effects was similar to the effects on the half-time tracer particle clearance. In conclusion, the dust overload at a lung burden of 3 mg test toner in rats was persistent for at least 15 months after termination of exposure.**

## Introduction

An impairment of alveolar clearance was observed after exposure to a high concentration of insoluble particles in previous subchronic and chronic inhalation studies in rats (*1,2*). In the same chronic study, an increase of polymorphonuclear neutrophils (PMN) in the bronchoalveolar lavage fluid (BALF) indicated inflammatory responses in the lung (*3*). These effects are some characteristic signs of lung overloading, a phenomenon that has been discussed by Morrow (*4*). The objective of the present study was to investigate whether these effects are reversible upon cessation of exposure. After a subchronic inhalation of a pigmented polymer (test toner) during a 15-month post-treatment observation period, the clearance of tracer particles, the number of cells in the BALF, and the level of some characteristic enzymes in the BALF were measured.

## Materials and Methods

A 9000-type xerographic toner material composed of about 90% styrene/l-butylmethacrylate random copolymer (CAS no. 25213-39-2) and 10% high-purity furnace-type carbon black (CAS no. 7440-44-0) was specially prepared for animal studies. Relative to commercial toner, the respirable fraction of particles was enriched about 10-fold according to the American Conference of Governmental and Industrial Hygienists criteria. The mass median aerodynamic diameter (MMADD) was 4.0 μm with a geometric standard deviation of 1.5. Female SPF F-344 rats were exposed 6 hr/day, 5 days/week for 3 months to this test toner at aerosol concentrations of 0, 10, and 40 mg/m$^3$. The retention of test toner in the lungs and in the lung-associated lymph nodes (LALN) was analyzed by a photometric determination after dissolving the lung tissue (*1*).

To allow a better insight into exposure effects on pulmonary clearance kinetics, γ-labeled particles were also administered which permit noninvasive measurements. The tracer aerosols of $^{59}Fe_2O_3$, $^{51}$Cr-polystyrene, and $^{85}$Sr-polystyrene with MMADDs of about 0.4, 0.76, and 3.8 μm and geometric standard deviations of 1.9, 1.5, and 1.26, respectively, were inhaled (nose only) for 0.5–1.0 hr by eight animals per group (*2*). The retained lung burden of tracer particles was below 1 μg. The thoracic γ activity was measured twice weekly. The rate coefficients of alveolar clearance were calculated from data for days 15–90 for each animal. After the 90-day period, animals were sacrificed and the γ activity of the lungs, the LALN, and the BALF was measured. The BALF was obtained by a first set of lavages with 2 × 4 mL saline and a second set of lavages with 4 × 5 mL saline and mild massage of the lung. A differential cell count of the BALF enabled the number of macrophages, polymorphonuclear cells (PMN), and lymphocytes to be determined. The distribution of test toner particles in macrophages was analyzed by light microscopy. After centrifugation of cells, lactate dehydrogenase (LDH), β-glucuronidase, and total protein were measured in the supernatant.

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At 3 months and 18 months of the study, five rats from each group were sacrificed for histopathology. Methods of animal sacrifice, tissue processing, and histopathology are described by Muhle et al. (*3*).

## Results and Discussion

The quanitity of test toner retained in the lungs at the end of exposure was 0.4 and 3.0 mg for the low and high exposure groups, respectively. After 15 months, the corresponding values were 0.12 and 2.65 mg in the lungs and about 5 and 190 $\mu$g in the LALN. From the retention data, overall half-times of toner clearance were calculated as 277 and 2845 days for the low and high exposure groups, respectively.

The results of BALF enzyme analysis, differential cell count, and tracer clearance are summarized in Tables 1 and 2. The absolute values are presented only for the control group, whereas for all exposure groups values are shown as percentages of the control values. In the high exposure group, LDH, $\beta$-glucuronidase, and total protein were significantly elevated, and only a minor recovery was observed 15 months after termination of exposure. For LDH and $\beta$-glucuronidase, the highest increase compared to controls was detected after 3 months. The differential cell count indicated only a slight increase in the number of PMN in the low exposure group but a substantial increase after high exposure. The number of lavagable macrophages were only slightly affected in both exposure groups.

The alveolar clearance of all three $\gamma$-labeled particles was slightly retarded at the low exposure level, but almost completely impaired at the high exposure level. The most pronounced impairment of the alveolar clearance of the polystyrene tracer particles occurred 3 months after termination of exposure to the

**Table 1. Pulmonary effects of a test toner inhalation: summary of enzymatic activities and differential cell count in the bronchoalveolar lavage fluid.[a]**

| | | | | Post-treatment observation period, months | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposure concentration, mg/m³ | | 0 | | 3 | | 6 | | 9 | | 15 | |
| Parameter | Group | | Unit | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| LDH | 1 | 0 | U/L | 32.5 | 7.2 | 27.3 | 5.7 | 27.0 | 5.0 | 25.2 | 6.2 | 38.2 | 38.0 |
| | 1 | 0 | % of control | 100 | 22 | 100 | 21 | 100 | 18 | 100 | 25 | 100 | 99 |
| | 2 | 10 | | 125 | 18 | 127 | 24 | 108 | 19 | 121 | 23 | 89 | 37 |
| | 3 | 40 | | 371* | 58 | 476* | 98 | 353* | 40 | 351* | 79 | 187 | 86 |
| $\beta$-Glucuronidase | 1 | 0 | U/L | 0.135 | 0.052 | 0.079 | 0.073 | 0.080 | 0.023 | 0.126 | 0.032 | 0.134 | 0.069 |
| | 1 | 0 | % of control | 100 | 38 | 100 | 93 | 100 | 28 | 100 | 26 | 100 | 51 |
| | 2 | 10 | | 101 | 32 | 86 | 72 | 109 | 49 | 104 | 30 | 105 | 38 |
| | 3 | 40 | | 752* | 124 | 1225* | 178 | 967* | 222 | 592* | 219 | 393* | 86 |
| Total protein | 1 | 0 | mg/L | 92.9 | 7.1 | 95.6 | 13.7 | 119.5 | 27.9 | 101.0 | 17.8 | 90.1 | 43.5 |
| | 1 | 0 | % of control | 100 | 8 | 100 | 14 | 100 | 23 | 100 | 18 | 100 | 48 |
| | 2 | 10 | | 131* | 10 | 118 | 20 | 93 | 14 | 104 | 18 | 98 | 25 |
| | 3 | 40 | | 215* | 18 | 220* | 33 | 169* | 20 | 181* | 31 | 159† | 36 |
| PMN | 1 | 0 | cells/mL | 778 | 668 | 406 | 397 | 650 | 686 | 230 | 318 | 1005 | 597 |
| | 1 | 0 | % of control | 100 | 86 | 100 | 98 | 100 | 106 | 100 | 138 | 100 | 59 |
| | 2 | 10 | | 663 | 218 | 434 | 150 | 240 | 163 | 470 | 513 | 89 | 44 |
| | 3 | 40 | | 14,140* | 3,317 | 17,913* | 3,777 | 8,075* | 2,289 | 16,107* | 3,606 | 4,311* | 769 |
| Macrophages | 1 | 0 | cells/mL | 187,397 | 18,905 | 193,134 | 23,768 | 192,625 | 26,734 | 191,429 | 15,238 | 241,536 | 47,556 |
| | 1 | 0 | % of control | 100 | 10 | 100 | 12 | 100 | 14 | 100 | 8 | 100 | 20 |
| | 2 | 10 | | 116 | 18 | 101 | 17 | 99 | 10 | 99 | 15 | 122 | 22 |
| | 3 | 40 | | 152* | 31 | 89 | 24 | 105 | 15 | 120 | 28 | 113 | 24 |

Abbreviations: LDH, lactate dehydrogenase; PMN, polymorphonuclear cells.
[a]Means and standard deviations are presented as absolute values for controls and as percentage of the control values for all groups.
*Significantly different from controls, Dunnett's test, $p < 0.01$.
†Significantly different from controls, Dunnett's test, $p < 0.05$.

**Table 2. Pulmonary effects of a test toner inhalation: summary of the clearance rate coefficient of inhaled tracer particles.[a]**

| | | | | Post-treatment observation period, months | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposure concentration, | | 0 | | 3 | | 6 | | 12 | |
| Parameter | Group | mg/m³ | Unit | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| $k$, $^{51}$Cr-polystyrene | 1 | 0 | 1/days | 0.021 | 0.001 | 0.018 | 0.001 | 0.016 | 0.001 | 0.012 | 0.001 |
| | 1 | 0 | % of control | 100 | 4 | 100 | 7 | 100 | 9 | 100 | 10 |
| | 2 | 10 | | 70* | 5 | 66* | 8 | 83* | 6 | 86† | 13 |
| | 3 | 40 | | 25* | 2 | 12* | 3 | 13* | 5 | 25* | 4 |
| $k$, $^{85}$Sr-polystyrene | 1 | 0 | 1/days | 0.015 | 0.003 | 0.015 | 0.002 | 0.018 | 0.003 | 0.009 | 0.003 |
| | 1 | 0 | % of control | 100 | 18 | 100 | 10 | 100 | 14 | 100 | 35 |
| | 2 | 10 | | 68* | 18 | 55* | 10 | 72* | 9 | 87 | 28 |
| | 3 | 40 | | 20* | 8 | 7* | 6 | 12* | 7 | 24* | 14 |
| $k$, $^{59}Fe_2O_3$ | 1 | 0 | 1/days | 0.014 | 0.002 | 0.014 | 0.001 | 0.013 | 0.001 | 0.011 | 0.001 |
| | 1 | 0 | % of control | 100 | 12 | 100 | 6 | 100 | 8 | 100 | 12 |
| | 2 | 10 | | 93 | 5 | 93 | 10 | 98 | 7 | 92 | 8 |
| | 3 | 40 | | 63* | 7 | 58* | 4 | 57* | 7 | 66* | 10 |

[a]Means and standard deviations are presented as absolute values for controls and as percentage of control values for all groups.
*Significantly different from controls, Dunnett's test, $p < 0.01$.
†Significantly different from controls, Dunnett's test, $p < 0.05$.

test toner. Thereafter the clearance impairment was reduced from a factor of about 10 to a factor of 4 at 12 months compared to control values.

In the high exposure group, the pattern of the effects during the 15-month post-treatment observation period was similar for β-glucuronidase activity, half-time of tracer polystyrene particles, and number of PMN in BALF. The observation of the persistent retardation of the alveolar clearance, even after a 12-month recovery period, was unexpected. During this recovery period, the particle distribution in the lung is changed. Histopathologically, aggregations of particle-laden macrophages were detected in focal areas, whereas in most areas of the lung, particle-laden macrophages were found only sporadically. The half-time for particle clearance of a few hundred days is much longer than the turnover time of an alveolar macrophage, which was estimated to be about 4 days in mice under the condition of no particle load (*5*). This means that there is a release of particles by dying macrophages and an immediate rephagocytosis by intact or newly arrived macrophages. At the high exposure level the percentage of macrophages without toner particles in the BALF increased from 25% at the termination of exposure to 85% after the 15-month observation period. Nevertheless, these macrophages were not able to remove recently inhaled tracer particles. A possible explanation is a release of chemotactic factors from particle-laden macrophages in areas of sequestration, which induce other macrophages containing labeled particles to remain in the vicinity of this area instead of being cleared to the upper respiratory tract.

## REFERENCES


1. Muhle, H., Bellmann, B., Creutzenberg, O., Fuhst, R., Kilpper, R., Koch, W., MacKenzie, J. C., Morrow, P., Mohr, U., Takenaka, S., and Mermelstein, R. Subchronic inhalation study of toner in rats. Inhal. Toxicol. 2: 341–359 (1990).
2. Bellmann, B., Muhle, H., Creutzenberg, O., Dasenbrock, C., Kilpper, R., MacKenzie, J., Morrow, P., and Mermelstein, R. Lung clearance and retention of toner, utilizing a tracer technique during chronic inhalation exposure in rats. Fundam. Appl. Toxicol. 17: 300–313 (1991).
3. Muhle, H., Bellmann, B., Creutzenberg, O., Dasenbrock, C., Ernst, H., Kilpper, R., MacKenzie, J., Morrow, P., Mohr, U., Takenaka, S., and Mermelstein, R. Pulmonary response to toner upon chronic inhalation exposure in rats. Fundam. Appl. Toxicol. 17: 280–299 (1991).
4. Morrow, P. E. Possible mechanisms to explain dust overloading of the lungs. Fundam. Appl. Toxicol. *10*: 369–384 (1988).
5. Bowden, D. H., and Adamson, Y. R. Role of monocytes and interstitial cells in the generation of alveolar macrophages: I. Kinetic studies in mice. Lab. Invest. 42: 511–517 (1980).