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# Effects in the Rat of Inhaling PVC Dust at the Nuisance Dust Level (10 mg/m$^3$)

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ABSTRACT. Rats inhaled a paste polymer polyvinyl chloride dust at an aerosol concentration of 10 mg/m$^3$ for 6 hr/day, 5 days/wk for a 15-wk period. A small number of randomly scattered lung lesions were detected at 15 wk; these lesions were also present 15 wk after exposure to polyvinyl chloride had ceased. Few if any biochemical changes were detected at the alveolar surface in lung tissues or other organs of polyvinyl chloride-exposed rats. It is therefore concluded that at "nuisance" dust level this form of polyvinyl chloride polymer exhibits a weak biological reactivity.

DURING ONE STAGE in polyvinyl chloride (PVC) manufacture the material exists as a fine, easily respirable dust. Consequently, investigators have examined the effects of this dust on mammalian lung.

The few experimental studies that have been reported present conflicting data on PVC toxicity. This may be due to the different formulations of PVC examined and/or differences of experimental designs. In the only previously reported inhalation study,[1] the authors suggest that continuous (24 hr) exposure of guinea pigs for 2-7 months in a PVC bagging plant resulted in the formation of granulomatous foci in the lung. The nature of the PVC used in that study—possibly a mixture of homopolymer and paste polymer—is not described, and the level of exposure is poorly defined.

Using a simple in vitro technique of hemolysis it has been shown that different formulations of PVC exhibit differential biological potential, as assessed by their ability to react with red blood cell (RBC) membranes.[2,3] Thus, homopolymers are not hemolytic, whereas paste polymers containing surfactants (detergents) on their particle surface produce extensive RBC damage in vitro. The chemical formulation of the detergent used in the preparation of the PVC polymer was, therefore, considered important in its hemolytic reaction.[3] Similar findings were reported by other investigators[4,5] who found that certain emulsion (paste) polymers were cytotoxic to rat peritoneal macrophages maintained in vitro, which is a test system considered by some investigators to be indicative of particle fibrogenicity in vivo. These investigators,[5] however, subsequently concluded that some paste polymers containing detergents produce a "false-positive" result in the in vitro system because the intratracheal 2-mg injection of the identical polymers into rat lungs produces no progressive fibrogenic response (unlike $\alpha$ quartz). All the polymers tested in experimental animals produce only a mild inflammatory response, and it was therefore concluded that PVC per se is an inert material.[5]

Intratracheal instillations of high doses (25 mg) of PVC have been shown to induce biochemical and histopathological changes in rat lung tissue;[6] other studies[7] have indicated that the cellular and biochemical effects at the alveolar surface and in lung tissue of instilled PVC particles are dose-dependent. However, aggregates of PVC dust given intratracheally, like many other materials given in this way, can produce a different, usually more vigorous, response from that produced by inhaled dust. Additionally, it is not possible to convert a single intratracheal instillation of a known volume of dust to a period of exposure at a multiple or fraction of the dust level, which means that for assessing safe occupational exposure levels, the results from intratracheal instillation studies are open to critical interpretation.

The aim of the present study was to determine whether the inhalation of a paste polymer, PVC-7, containing the detergent sodium dodecyl sulphate,[3,7] at "nuisance" dust level (10 mg/m$^3$) would produce any alteration in the lungs of experimental animals. The parameters chosen for investigation included a study of the biochemical integrity of the alveolar surface by estimation of free cell number and enzyme activities, and determination of pulmonary surfactant levels and soluble protein. In addition to histopathological examination of the lung, DNA, and protein 'synthesis' and hydrolytic enzyme activity of alveolar tissue were monitored.

## MATERIALS AND METHODS

Animals. Eighty 10-wk-old female albino rats of the Sprague Dawley C.D. strain (Charles River, U.K. Ltd., Margate, Kent) were used in this study. These were randomly allocated to either the control or PVC-exposed group so that each group consisted of 40 rats. The rats were housed in groups of four, in polypropylene cages with stainless steel mesh floors. Food and water were supplied ad libitum while the rats were in the cages. The room temperature was maintained at 20 ± 2°C, and lighting was controlled to provide a 12-hr light period each day.

Aerosol exposure. A paste polymer, PVC-7, which contains sodium lauryl (dodecyl) sulphate surfactant was used throughout the study. The PVC-treated animals were exposed to a mean aerosol concentration of 10 mg/m$^3$, i.e., "nuisance" dust level (Guidance Note EH 15/77, Health and Safety Executive), on 5 days for 6 hr/day for 5 days/wk up to 15 wk. Groups of 10 exposed rats were examined with control animals at 3, 9, and 15 wk after the experiment was initiated. One group of animals was maintained without further contact with PVC for 15 wk after being previously exposed to the dust for 15 wk. A nonexposed control group was also maintained during this time period.

Exposures were carried out in a perspex, cubic, 240-L chamber which was operated under dynamic conditions at a slight positive pressure. The aerosol of PVC particles was generated using a Wright dust feed mechanism (L. Adams Ltd.; Minerva Road, Chase Estate, London NW10). Following preliminary experiments to determine optimum operating conditions, a dust packing force of 0.2 tons was used. The mechanism was operated with standard canister liner at a gear ratio of 4 : 1 reduced. Total chamber air was supplied through the dust feed which was run at 1.05 kg/cm$^2$ (25 L/min). The aerosol was introduced into the exposure chamber at the base center and then ascended through an area clear of holding cages to descend through the cages in which the animals were individually housed, and left the chamber through a series of holes at the base. The chamber was held in a fume cupboard which was at negative pressure with respect to the laboratory, and the exhausted dust was removed by an absolute filtration unit. Control animals were placed in an identical chamber, but were exposed only to the same compressed air as that used to operate the dust feed mechanism. Aerosol concentration was determined twice daily by gravimetric analysis of samples collected with an openface filter holder operated in the vertical position. Due to the extremely low aerosol concentration, a sampling rate of 10 L/min was required to ensure accurate assessment of total chamber concentration. The particle size distribution in the chamber was measured using an Anderson mini-sampler.

Treatment of animals prior to biochemical analyses. In the biochemical studies six exposed and six control rats were examined at each chosen time interval and treated identically.

Each animal was given an intravenous tail vein injection of 75 $\mu$Ci of [methyl-$^3$H]-thymidine (52 Ci/mmol) and 2.5 $\mu$Ci of L-[U-$^{14}$C]-proline (282.5 mCi/mmol) and left

Table 1.—Cellular and Biochemical Studies on Rats Exposed to 10 mg/m$^3$ PVC Dust for Different Time Periods

| Exposure Period/ Animal Group | Lung Weight/ Body Weight Ratio × $10^2$ | Number Free Cells/Animal × $10^{-6}$ | Pulmonary† Surfactant mg/Animal | Pulmonary† Lavage Protein mg/Animal | Acid RNAase units/$10^6$ Cells | Acid RNAase units/g Lung | DNA mg/ Lun |
|---|---|---|---|---|---|---|---|
| 3 Weeks | | | | | | | |
| Control | 0.53 (±.086) | 6.71 (±0.93) | 0.50 | 2.25 | 0.68 (±0.12) | 74 (±10) | 4.54 (±0.76) |
| PVC-Exposed | 0.55 (±.082) | 8.86 (±1.52) | 0.52 | 2.62 | 0.69 (±0.29) | 75 (±19) | 4.06 (±0.43) |
| 3 Weeks | | | | | | | |
| Control | 0.47 (±.036) | 19.35 (±4.64) | 0.60 | 3.19 | 1.17 (±0.27) | 108 (±12) | 4.93 (±1.07) |
| PVC-Exposed | 0.48 (±.037) | 13.79 (±4.63) | 1.50* | 3.96 | 1.39 (±0.31) | 104 (±12) | 5.30 (±0.87) |
| 15 Weeks | | | | | | | |
| Control | 0.43 (±.023) | 11.00 (±3.61) | 0.66 | 2.86 | 0.90 (±0.31) | 83 (± 5) | 4.01 (±0.87) |
| PVC-Exposed | 0.40 (±.045) | 11.00 (±1.08) | 0.62 | 3.11 | 1.10 (±0.21) | 104 (±16)* | 4.78 (±0.32) |
| 15+ Wk | | | | | | | |
| 15 Wk Clearance | | | | | | | |
| Control | 0.39 (±.028) | 9.26 (±2.93) | 1.28 | 3.50 | 0.96 (±0.22) | 45 (± 8) | 4.23 (±0.25) |
| PVC-Exposed | 0.41 (±.063) | 11.59 (±1.97) | 1.20 | 4.13 | 0.72 (±0.23) | 50 (± 9) | 4.27 (±0.29) |

NOTE: Numbers within parentheses refer to standard deviation.

*Significantly different from control. + Values represent mean of pooled samples from six rats.



for exactly 1 hr. They were then sacrificed by intraperitoneal pentobarbitone injection, a blood sample was then removed from the hepatic portal vein, and the lungs perfused via the heart with 0.15 *M* NaCl to remove blood from the vascular bed. The lungs were removed from the pleural cavity and lavaged six times with 0.15 *M* NaCl, after which they were dried between paper towels and weighed. The lavaged lung tissue and pooled washes were kept on ice prior to further treatment (see below). Other tissues taken for examination were the liver, spleen, kidney and sections of the gastrointestinal tract which were stored frozen until required.

**Fractionation of lavage fluid and biochemical analysis.** The free cell population from each animal lavage was obtained by centrifugation at 300 *g* for 20 min at 4°C. The supernatant fraction from six rats in each group was pooled and centrifuged at 1000 *g* for 1 hr at 4°C. The supernatant fraction from this centrifugation contained the majority of soluble alveolar lavage protein.[8] The pellet was resuspended in 4 *M* NaCl, mixed well, and centrifuged at 1500 *g* for 25 min at 4°C, and the resulting separation gave a pellicle of lipoprotein-rich material, designated pulmonary surfactant, which floated to the top of the tube. This was collected, dialyzed against distilled water, freeze-dried, and weighed.[9] The free cell population was counted and the levels of acid RNAase and acid protease were determined as described previously.[9] The incorporation of [$^3$H]-thymidine into tissue DNA and [$^{14}$C]-proline into tissue protein was determined as follows. Body tissues were homogenized in 0.15 *M* NaCl and samples suspended in a final concentration of 0.2 *M* perchloric acid (PCA) for 1 hr. The mixture was centrifuged at 1000 *g* for 20 min. and the supernatant, containing free label, discarded. This procedure was repeated once more and the resulting pellet was then resuspended in 0.5 *M* PCA at 70°C for 20 min. to solubilize the DNA. The supernatant derived from centrifuging this mixture at 1000 *g* for 20 min. was stored and the process repeated. The supernatant fractions were pooled and samples taken for chemical analysis of DNA and direct counting of [$^3$H] label. The remaining pellet was suspended in distilled water and samples digested in 1 *M* NaOH to assay for protein content or taken up in Soluene for determination of [$^{14}$C] radiolabel. The efficiency of counting was determined by using internal standards and results were expressed as disintegration per minute of incorporated radiolabel per mg DNA or protein for each tissue examined.

**Histopathology.** The trachea and lungs from four control and four PVC-exposed rats were removed after the rats had been killed by exsanguination under deep pentobarbitone anaesthesia. The lungs were infused to constant pressure (10 cm water) with buffered 10% formalin, and

| $^{3}$[H]-Thymidine in Lung DNA dpm × $10^{-3}$/mg DNA | Protein mg/g Lung | $^{14}$[C]-Proline in Lung Protein dpm/mg Protein |
|---|---|---|
| 7.5 (± 4.5) | 50.6 (± 8.52) | 164 (±55) |
| 10.4 (± 3.6) | 52.0 (± 7.46) | 165 (±47) |
| 40.0 (±48.0) | 77.3 (±19.72) | 160 (±23) |
| 25.6 (±14.2) | 87.7 (±10.68) | 120 (±23)* |
| 14.3 (± 6.9) | 70.9 (±10.50) | 156 (±20) |
| 10.3 (± 2.3) | 77.5 (± 4.14) | 165 (±21) |
| 7.7 (± 2.4) | 80.5 (± 6.84) | 94 (±33) |
| 7.5 (± 1.9) | 91.2 (±11.58) | 74 (± 6) |

Fig. 1. Lung from rat exposed to PVC dust for 3 wk. Transmission electron microscopy of alveolar macrophage containing numerous smooth-surfaced particles (*arrows*) identical to the inhaled PVC dust (scale unit = 2 μm).

transverse sections prepared from embedded material. Sections were stained with hematoxylin/eosin, Masson's trichrome stain for collagen,[10] silver stain for reticular fibers,[11] and with modified Sudan IV to identify PVC particles.[12] Some material was also processed for electron microscopy.

## RESULTS

Body weight and clinical signs. There was no effect on body weight of PVC-exposed rats nor any clinical signs that could be related to PVC exposure during the study.

Chamber aerosol analyses. The chamber aerosol was maintained at an average concentration of 10.6 mg/m$^3$ [standard deviation (*SD*) = 2.3, *N* = 146]. The highest and lowest concentration recorded was 15 mg/m$^3$ and 7 mg/m$^3$, respectively. The mass median aerodynamic diameter of the aerosol was 1.7 μm (σg 4.64).

Biochemical studies. Very few significant changes in lung weight/body weight ratio, pulmonary surfactant and alveolar surface protein, free cell number and enzyme activities or lung enzyme activities, protein and DNA 'synthesis' were detected in animals exposed to PVC dust throughout this study (Table 1). In addition, DNA and protein 'synthesis' in the liver, spleen, and kidney, together with changes in acid protease levels in the free cells and lavaged lung tissue, were not detected in PVC-exposed rats (data not shown). Three significant changes in rats exposed to PVC were found: (1) elevated pulmonary surfactant, (2) depressed lung protein 'synthesis' at 9 wk, (3) elevated lung acid RNAase activity at 15 wk.

Histopathology. At 3 wk the lungs of both control and PVC-exposed rats appeared normal, except that in the exposed group the alveolar macrophages were enlarged and contained numerous, 1-4 μm diameter, smooth-surfaced particles similar in appearance to PVC dust (Fig. 1). At 9 wk there was evidence of a mild respiratory tract infection in both control and PVC-exposed groups characterized by increased numbers of mononuclear cells in the perivascular spaces, and in most animals, an increase in the volume and extent of bronchus-associated lymphatic tissue. The foamy macrophages seen at 3 wk in PVC-exposed animals were less evident, possibly because of an increased turnover of cells in the 9-wk group. At 15 wk there was no sign of the respiratory tract infection seen in the rats at 9 wk. In the PVC-exposed animals there were aggregates of foamy macrophages occupying groups of up to 9 alveoli; also associated with these aggregates was hypercellularity due to an increase in mononuclear cells and fibroblasts, in the interstitium of the alveolar wall (Fig. 2). This interstitial reaction was also associated with minimal increase in collagen and reticular fibers. The smooth-

Fig. 2. Lung from rat exposed to PVC dust for 15 wk. Note aggregation of foamy macrophages and hypercellularity of adjacent alveolar wall (*arrows*). Hematoxylin and eosin stain (X 250).

surfaced particles that filled foamy macrophages stained red with modified Sudan IV, indicating that they were PVC dust.[12] Fifteen weeks after the final exposure (30 wk from commencement of the experiment), the lungs of control animals appeared normal, while those previously exposed to PVC showed no significant change in the lesions detected at 15 wk (termination of exposure).

## DISCUSSION

The results show that a paste polymer preparation of PVC dust inhaled by rats at "nuisance" dust level (10 mg/m$^3$) for 6 hr/day, 5 days/wk, produces small, randomly scattered, lung lesions after 15 wk of exposure. These lesions are characterized by hypercellularity of the interstitium of the alveolar walls in areas adjacent to macrophage aggregates containing PVC. In addition, the lesions persist 15 wk after the cessation of animal exposure to the particulate. The PVC-induced lesions, however, show only minimal increase in collagen and reticular fiber formation, with no evidence of extensive fibrotic reaction. Similar conclusions are reported by other investigators[5] who studied the effects of intratracheal instillations of different PVC formulations.

The results of the current histopathological study therefore suggest that at "nuisance" dust level PVC has a relatively weak biological reactivity, and this conclusion receives further support from the biochemical investigation. While PVC-exposed animals have elevated levels of pulmonary surfactant and show a decrease in lung protein 'synthesis' 9 wk after exposure, the presence of a mild respiratory tract infection in all the animals during this time period prevents the establishment of any definite conclusions. Thus relatively few, if any, biochemical changes in PVC-exposed rats are detected at any exposure period—either at the alveolar surface, the lung tissue, or other body

In contrast to previous inhalation [illegible] exposed to 12 mg/m³ chrysotile asbestos for 5 days/wk for 15 wk have elevated free cell numbers (2-3 X control animals) free cell and lung enzyme activity (3-10 and 1-8 X control, respectively), and pulmonary surfactant (12 X control).[9] Elevations in pulmonary surfactant and free cell numbers are also detected in rats inhaling amosite asbestos and fiber glass (12 mg/m³, 5 days/wk, for 8 wk).[13] With dusts of "high" biological reactivity these biochemical changes at the lung surface persist with the progressive pathological development of lung disease.

In summary, the present short-term study indicates that at "nuisance" dust level one form of paste polymer PVC has a weak biological reactivity, only detectable by histopathological examination, which reveals a small number of lung lesions in experimental animals. It is not possible at this time to interpret this finding in terms of the lesion likely to arise in man under similar conditions of exposure.

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