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INFLUENCE OF POLYVINYL CHLORIDE (PVC) DUST ON RAT RESPIRATORY SYSTEM

by

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RECEIVED
AUG 6 1979
R. N. WHEELER, JR.

Your ref.	Our ref	Tel ext	Date
	JS/AM/DSO-107	3162	20 July 1979

Dear Nick

PVC DUST

Thank you very much for your letter of July 6.

I have pleasure in enclosing our translation of the paper by Popow which you requested. I do not know whether this will be of any interest to you in the liability case which you have. As you will see when you read it, Popow's experimental conditions were utterly extreme. I think any biological system will be overwhelmed by 97 g/m^3 of PVC or any other fine dust. These conditions are a long way from our TLV of 10 mg/m^3.

I have just got back from my annual vacation and your letter was on the top of my pile. I have not given copies of this Popow translation to Ted Torkelson, Maurie Johnson or any of my other American friends. It may be some little time before I get around to doing this. If you could run off some quick copies for them, I would be grateful.

With best wishes

Yours sincerely

J Stafford
Division Manager
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Enc

20-39009

C O N T E N T S

I. INTRODUCTION

The pathological changes occurring in the respiratory system under the influence of inspiration of air contaminated with particles of various dusts have been described for about 100 years as dust disease - pneumoconiosis. Initially pathogenic action was only ascribed to silicon dust. Therefore the investigations undertaken mainly concerned silicosis. On the basis of clinical studies (Levin [73], Sokołow et al. [109], Vigliani et al. [122]) and experimental studies (Cabański et al. [13], Gross et al. [37], Policard et al. [95]) it was found that in the lungs silicon dust causes formation of fibrous nodules, composed of thick strands of collagenous fibres, undergoing hyalinization. Sometimes there are necrotic masses at the centre of such a nodule. The discovery of the pathogenic properties of silicon dust had the result that many authors turned their attention to the problem of dust diseases. Clinical and experimental investigations made it possible to determine the action of individual types of dust. As a result of this research it was established that coal dust accumulates in the lungs, damages the pulmonary alveoli and causes formation of dust nodules with the character of giant-cell granulomas. These nodules are of a constant character with susceptibility to fibrous atrophy (Niepołomski et al. [80], Schepers [106], Worth et al. [121], Sośnierz et al. [110-112]). However, talc dust causes so-called intraparenchymatous focal pneumonia, in which collagenous fibres appear at a very late stage in the inflammatory foci and there is no formation of pneumoconiotic nodules (Rakowski [100]). In the course of asbestosis we can distinguish an early stage, called the vaso-histiocytic stage, and a late stage, called the fibroblastic-collagenic stage (Avril [3]). A characteristic feature of this coniosis is diffuse fibrosis and hyalinization without formation of nodular lesions. Sometimes so-called asbestos bodies

can be detected among the striae of connective tissue (Hames [40]). Cotton dust causes chronic bronchitis (Fetisova [25]), whereas cement dust causes acute tracheitis and bronchitis, and then chronic atrophic inflammation of these regions. In the lungs there is development of what is known as catarrhal-intraparenchymatous inflammation, leading to atrophy of the inter-alveolar septa. Fibrosis is not very pronounced and is of a constant nature (Niepołomski et al. [81]). Berylliosis is included among the toxic dust diseases in view of the mechanism of its development, and in the early stages there is exudative pneumonia, which quite quickly changes to a productive phase. There is formation of nodules composed of histiocytes and lymphocytes. These nodules are often surrounded by striae of collagenous fibres, undergoing hyalinization. Sometimes giant cells, similar to Langhans cells, are to be found in the centre of such a nodule (Marchand [75], Rüttner et al. [104], Stofer [114]).

There is no doubt concerning the existence of coniosis as a distinct nosologic unit (Kleczeński [61], Zahorski [123]). Research undertaken so far has endeavoured to establish the mechanism of its genesis (Amoudru [1], Ottowicz [93]). Many authors (Ebert et al. [24], Hames et al. [40], Heppleston [44], Policard [95]) consider that pneumoconiotic changes in the lungs only develop with participation of living cells. In the opinion of the above authors these cells absorb many particles of dust and then carry them to the connective tissue around the vessels, to the wall of the bronchi and alveoli and to the lymphatic vessels. However, there are those (Gross [36, 37]) who assert that the dust particles reach the connective tissue without the participation of living cells, as a result of the respiratory movements of the lungs. In recent years the literature has contained reports on the possibility of pathologic changes developing in the respiratory system reminiscent of coniosis, but caused by the action of plastics

(Boitsov [10], Roussel [102]). These reports drew my attention to plastics which are now in general use and have entered many spheres of daily life (Markiewicz [76], Kastierina et al. [57], Porejko et al. [97], Schildknecht [107]). On account of their plasticity, resistance to the action of acids and alkalies, ease of shaping and relatively low production costs, plastics products have also found application in biology and medicine [Frankowski [29], Jasiński et al. [53], Kawecki [58], Kawecki et al. [59], Kuś et al. [69, 70], Nowak [83, 84, 85, 87], Oleński et al. [89], Rob et al. [101], Rzepecki [105], Staniszewska [113], Szymańska et al. [116], Grabowski et al. [35], Kędra [60], Komczyński et al. [64]).

One of the most important and first synthetic thermoplastic macromolecular compound produced on an industrial scale was polyvinyl chloride (PVC) (Franty et al. [30], Wanderberg [119]). Pure polyvinyl chloride has the form of a white, tasteless and odourless powder. In industrial production, material made from it can be given various forms, with varying elasticity and hardness. Therefore these polyvinyl chloride products have found application in the meat industry (Bohosiewicz [9]), pharmaceuticals (Chwiałkowska et al. [15]), in sport (Kopczyński [65]) and in medicine (Goetzen [34], Homrowski et al. [47, 48], Jankowska [52] and others).

The introduction of various new chemicals and various products made from them in daily use has given rise to the problem of the action of these compounds on the human body, both of the producers and of the users. This problem has been dealt with in many works in the national and foreign literature (Cristea et al. [16], Bartenov et al. [4], Hervieux [45], Kalinin [54], Kalińska [56], Koelsch [62], Lachnit [71], Lefaux [72], Markiewicz [76], Moeschlin [78], Kowalska et al. [66], Kalinowska et al. [55], Smolik [108], Troszin [117], Cołow et al. [17], Deyanova et al. [20]). The problem of the toxicity of plastics, and especially polyvinyl chloride, is

still open, despite the fact that various investigations in this direction have been undertaken (Quooss [99], Russel [103], Pennarola et al. [94]). The opinion expressed in the vast majority of published works is that polymers are physiologically inert compounds, and the harmful effects are caused by auxiliary chemicals in the polymer manufacturing process (initiators, catalysts, emulsifiers) or taking part in forming the physical properties of the polymers (stabilizers, plasticizers) (Danishevskii et al. [19], Nowak [82]). Some authors (Filatova [26, 27], Gervais [32], Witnauer et al. [120]) ascribe harmful action to vinyl chloride, the toxic properties of which are already recognized and measures to prevent poisoning have already been taken in many factories (Beusnel [7], Broitman [11], Bugajska [12], Dmitreva [21], Gabor [31], Günther [39], Krivoglaz et al. [68]). However, many authors consider that certain plastics may have pathogenic properties and as confirmation of their viewpoint they cite the harmful effects of massive inhalation of polyamide dust. This dust causes acute catarrhal pneumonia with formation of granulomas of the type around foreign bodies (Giovacchini [33]), and sometimes even changes of the nature of storage disease (Medve et al. [77]). There are also those who ascribe carinogenic action to plastics (Fitzhugh [28], Hueper [51], Nowak [88], Nesswetha et al. [79], Oppenheimer et al. [90], Russel et al. [103], Bates et al. [5], Druckrey et al. [22, 23], Oppenheimer et al. [89]). As a result of these investigations it was found that after implantation of plates there is nearly always appearance of neoplasms [Guess et al. [38]), after implantation of a thread they only develop occasionally (Kogan et al. [63]), and neoplasms are never found after implantation of powder (Oppenheimer et al. [92]).

Although most authors are of the opinion that polyvinyl chloride is a physiologically neutral compound, in recent years the literature has

contained occasional reports on the occurrence of various bodily disorders and changes in the organs of workers employed in factories manufacturing polyvinyl chloride or products made from this material, viz: in the liver (Pushin [98]), especially in older women (Troshina [117], in the phalangeal bones of the hands and in the skin (Harris and Adams [42]), and in the vascular and nervous system (Suciu et al. [115]). Bugajska et al. [12] have drawn attention to the possibility of toxic action of PVC. Roussel et al. [102] found posthumous atypical changes in the lungs and were unable to assign these changes to a set of known nosologic units. These authors assume that these changes should perhaps be associated with work done for many years by this worker in a PVC factory. In addition to descriptions of clinical observations, the literature also contains experimental works conducted with polyvinyl chloride. Bernt (quoted by Danishevskii [6]), for example, fed animals an oily suspension of ground PVC and showed that it does not have any toxic properties, but he also ascertained that the material is soluble in the gastric and intestinal juice. Boitsov [10], however, mentions the possibility of development of coniosis in animals after intratracheal administration of PVC resin. He found that in their lungs there is thickening of the interalveolar septa and also peribronchial and perivascular inflammation. My preliminary investigations on rats (Popow [96]) submitted to the action of polyvinyl chloride also indicated that this dust is a noxious agent for the lungs. Lymphocyte-like cells multiply under the influence of inspiration of this dust. They accumulate in the form of cuffs around the blood vessels, and there is also intumescence of the muscles and proliferation of collagenous fibres in the wall of the bronchi, and pulmonary emphysema.

II. PURPOSE OF THE WORK AND ASSUMPTIONS

As follows from this review of the literature, the problem of the effect of plastics on the living organism has not yet been solved completely. Because polyvinyl chloride is one of the most frequently used plastics, we decided to conduct investigations of the respiratory system after long-term action of polyvinyl chloride and establish the nature of the resulting pathomorphological changes. Moreover, we tried to determine whether the changes in the lungs after cessation of exposure to PVC dust recede, whether they stop developing, or whether they still occur. Another interesting problem worthy of consideration is whether the changes occurring in the lungs promote the development of complications, as occurs for example in the course of silicosis. In the literature available to me I have not come across reports on this kind of experimental investigations on animals.

III. RESEARCH TECHNIQUE

The experiments were conducted on 60 white rats of both sexes of the Wistar strain. A control group comprised 10 rats. The age of the animals at the start of the experiment ranged from 3 to 4 months. The rats weighed from 190 to 220 g. The animals remained in separate cages, in a well-lit room which was aired every day, with its temperature maintained in the range from 16° to 20°C. The animals were given mixed, nonstandard food regularly. The test animals were divided into three groups.

Group I comprised 16 animals exposed to PVC dust from 1 to 4 months. In this group I observed the picture of all pneumoconiotic lesions. Group II comprised 22 rats, exposed to the dust from 5 to 12 months. In this group I observed the picture of late pneumoconiotic lesions. Group III comprised 22 rats which, after 12 months of exposure to the dust, were kept under observation for a period of 8 months. This group was employed for

investigating the behaviour of the pathologic changes that arose under the influence of polyvinyl chloride after its action had ceased. The control group comprised 10 animals. They were kept under observation for 20 months, 2 rats being killed by decapitation every 4 months.

Exposure to polyvinyl chloride dust was effected in the following way: the rats were placed in a special chamber with dimensions 1.46 m x 0.56 m x 0.38 m and were submitted to the action of polyvinyl chloride dust every day for 1 hour. A rubber hose was led into this chamber, and the other end of the hose was in a glass vessel with capacity of 2000 ml (mixer). Every day 30 g of PVC powder was poured into the mixer once. The mixer was connected by another rubber hose to an electric blower. When the blower was switched on, the PVC powder was mixed with air. Then using the outgoing rubber hose, the PVC-air mixture was led into the chamber where the animals were. The concentration and size of the PVC dust particles were monitored with a Zeiss coniometer during exposure to the action of the dust. At the start of the experiment, on average about 9000 dust particles were found in 1 mm^3. As time passed, this figure gradually fell, as follows (figures given after rounding off): 7000 dust particles after 15 minutes, 5000 dust particles after 30 minutes and about 1000 dust particles in the final phase of dust exposure. The size of the dust particles was as follows: smaller than 1 μm - 1%, less than 5 μm - 92%, from 5 μm to 10 μm - 4%, larger than 10 μm - 3%. The concentration by weight was 97 g/m^3. After exposure to the dust for 1 hour the rats were put back in the cages, where they remained for the rest of the time. The control group of animals was placed in the chamber for 1 hour every day and movement of air was created with the aid of the electric blower, but PVC was not introduced. All the animals were killed by decapitation.

From each animal lung segments were taken from the perihilar and subpleural region for microscopic investigation. The segments taken were fixed in Carnoy fluid, embedded in paraffin blocks and stained: hematoxylin and eosin, orcein according to Weigert's method for elastic fibres, according to Gomori's method for argentaffine fibres, according to Heidenhain's modification of Mallory's method (with "azan") for collagenous fibres, according to Turnbull's method for divalent iron and Perls' method for trivalent iron and with mucicarmin for the presence of mucus. In addition the following histochemical tests were effected: paS for neutral mucopolysaccharides (supplementing it with control acetylation according to Gersh and employing blocking with dimedon), tripaS for acidic protein and Hale's test for acidic mucopolysaccharides. In addition, staining with toluidine blue at pH 3.4 and 5.6 was effected for the purpose of investigating metachromasia and detection of mast cells. Polyvinyl chloride, designated by the symbol ED, was obtained from the Zakłady Chemiczny (Chemical Works) in Oświęcim. The results of the microscopic investigations relate to lung segments taken exclusively from animals that were killed at the scheduled time.

IV. RESULTS OF INVESTIGATIONS

1. CONTROL GROUP

Macroscopic picture. The animals in this group were killed at 4-monthly intervals. On dissection, in all the rats the lungs collapsed slightly after opening the thorax, and they were pale pink and airy on the external surface and on section. During cutting they crackle in the characteristic

way. No bronchiectasia and no nodular foci were found in the lungs of these animals.

Microscopic picture. The pulmonary alveoli are fairly regular, the interalveolar septa are of identical thickness with occasional slender elastic fibres (orcein staining). Around the thin-walled capillary vessels in the interalveolar septa we observed some lymphocyte-like cells (Fig. 1). The basement membrane of the capillary vessels contains slender thread-like collagenous fibres (staining with Azan) and slender argentaffine fibres (staining by Gomori's method), but do not contain paS-positive bodies, and stain yellow in the tripaS test.

The lobar bronchi are lined with simple cylindrical epithelium, which is multirowed with occasional beaker cells, and the ciliary apparatus is retained. In the cytoplasm of the beaker cells there are occasional, extremely minute mucus granules (staining pink with mucicarmin) and also paS-positive bodies (staining reddish-violet). Neither the cytoplasm of the cylindrical cells nor of the beaker cells contains acidic mucopolysaccharides (Hale's test proves negative). In the wall of these bronchi the muscle fibres run across circuitously in bundles and are separated by collagenous fibres (staining with Azan). The elastic fibres also run circuitously, slightly undulating (staining with orcein), and argentaffine in the form of tangles (staining by Gomori's method). Furthermore, in the wall of the bronchi there are a few lymphocyte-like cells and histiocytes. In the submucous membrane we can observe occasional glandular tubes with narrow lumen, containing neither mucus nor mucopolysaccharides (staining with mucicarmin; paS, tripaS and Hale's tests prove negative).

The lumen of the segmental bronchi and bronchioles is lined with cylindric cells, but beaker cells are not found among them (Fig. 1). The cytoplasm of the cylindric cells stains yellow in the tripaS test and does

not contain any granules: neither of mucus, nor of mucopolysaccharides (staining with mucicarmin and the paS and Hale's tests prove negative). The wall of the segmental bronchi has structure similar to the lobar bronchi.

No phagocytes with granules of haemosiderin were found in the cytoplasm in the rats of the control group. Occasional mast cells appear under the pleura (having fine-granular, reddish-violet cytoplasm when stained with toluidine blue).

2. GROUP I. EARLY CHANGES (RATS Nos. 1-16, PERIOD OF EXPOSURE TO DUST - 4 MONTHS)

Among the 16 animals in this group, 2 rats (Nos. 1 and 2) died at the start of the 4th month of experiment. Pulmonary emphysema and a considerable degree of passive congestion are found in all internal organs on dissection. A further 2 rats (Nos. 3 and 4) died in the last days of the 4th month of exposure to the dust. The macroscopic picture was similar to that of the 2 previous rats. The surviving 12 rats (Nos. 5-16) were killed

there is neither formation of fibrous-hyaline nodules, as is observed in silicosis, nor formation of giant-cell granulomas, undergoing fibrosis and atrophy, which are characteristic of anthracosis. We also did not observe the diffuse fibrosis and hyalinization that occurs in asbestosis. After long-term application of PVC dust there is development of atrophic bronchitis like that observed in cement pneumoconiosis. Mention should also be made of the differing behaviour of the pathomorphologic changes in the respiratory system upon cessation of exposure to the dust. In pneumoconiosis caused by polyvinyl chloride the connective tissue in the wall of the dilated bronchi is not cicatrized, and does not hyalinize around the bronchioles. Infiltrates of lymphocyte-like cells and histiocytes remain in the wall of the lobar and segmental bronchi. All this indicates a repair reaction. In pneumoconiosis caused by PVC dust, proliferation of lymphocytes and growth of bronchial glands is more intensive than in berylliosis. It follows from all these comparisons that pneumoconiosis caused by PVC dust has a distinct morphologic picture. This is further emphasized by the fact that the pathomorphologic changes described in the respiratory system in the course of pneumoconiosis caused by polyvinyl chloride dust develop more slowly. According to Pushin [98], this is typical of diseases caused by macromolecular compounds.

Comparing the results of the changes caused by PVC dust in the respiratory system of rats, the opinion may be expressed that this dust causes pneumoconiosis of a toxic nature. It is also necessary to discuss the fairly large group of animals which died in the course of exposure to the dust. Four rats died in Group I - in all of them, in addition to pulmonary emphysema and bronchial catarrh, we found a marked degree of passive hyperaemia in the internal organs. This might indicate circulatory insufficiency, probably existing prior to the commencement of exposure to the PVC dust,

which increased under the influence of the mechanical and toxic action of this dust and led to the animals' deaths. Ten animals died in Group II. In all of these, apart from the signs of circulatory insufficiency we ascertained bronchiectasia in a suppurative state and pulmonary suppuration. In Group III, six rats died in the first month after cessation of the action of the dust. The pathomorphologic changes in the lungs determined during dissection were similar to the changes described in Group II. All the animals still alive survived for a further 7 months. In my opinion the death of animals before the intended time of observation can be explained by the existence of lesions which were intensified under the influence of the polyvinyl chloride dust. It would have been difficult for me to notice these existing lesions prior to commencement of the experiments.

VI. CONCLUSIONS

The following conclusions can be drawn on the basis of these experiments and analysis of the pathomorphologic changes produced in the respiratory system of rats exposed to polyvinyl chloride dust:

1. Polyvinyl chloride dust introduced into the respiratory system is biologically active, exerting mechanical and toxic action, and producing lesions of the nature of toxic pneumoconiosis.

2. The pneumoconiotic lesions are based on:

   a) development of bronchial catarrh and bronchiectasia with the appearance of paS-positive bodies in the cells of their epithelium,

   b) wart-like hyperplasia of components of the bronchial wall with formation of nodular foci similar to cystadenomas,

c) multiplication of lymphocyte-like cells and histiocytes in the bronchial wall and aggregation of these cells in the form of cuffs around the blood vessels in the interalveolar septa,

d) development of pulmonary emphysema and chronic inflammation around the bronchioli.

3. Development of pneumoconiosis caused by polyvinyl chloride can be divided into two stages: catarrhal-degenerative and hyperplastic-inflammatory.

4. The pneumoconiotic lesions develop very slowly, are different from other known conioses and create favourable conditions for suppurative complications, which mainly affect bronchiectasia.

5. Some of the pathomorphologic changes in the lungs arising in the course of experimental coniosis caused by polyvinyl chloride dust do not display any marked tendency to recede after cessation of exposure to this dust.

VII. SUMMARY

(In Polish, English and Russian)

FIGURE CAPTIONS are given in Polish and English.

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