IN-VITRO STUDIES OF A RANGE OF FORMULATIONS OF PVC

by

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INTRODUCTION

The polymer PVC is generally considered as biologically inert, although there are isolated reports in the literature of lung changes in PVC workers (SZENDE et al 1970, VERTKIN & MANONTOV 1970, LILIS et al 1975). Earlier work in this laboratory (STYLES & WILSON 1973) demonstrated neither in-vitro cytotoxicity of PVC or fibrosis in rats given an intraperitoneal injection of polymer. However FRONGIA, SPINAZZOLA & BUCARELLI (1974) reported pulmonary changes in guinea pigs and rats continuously exposed to a high concentration of PVC dust. More recently RICHARDS et al (1975) reported high haemolytic activity in a sample of PVC dust, though a second sample proved essentially inert. This latter result suggests the possibility of differential effects attributable to the varying formulations of PVC required to produce materials for different end usages. It was therefore decided to subject a range of formulations of PVC to an in-vitro assessment of cytotoxicity, and to test representative samples of the various grades in whole animal studies. The results of the in-vitro tests are summarised in this paper.

METHODS AND MATERIALS

The method used to assess cytotoxic potential of dusts was essentially that of Styles & Wilson (1973) using peritoneal macrophages. 200-400 gm SPF Wistar rats (Alderley Park Strain) of both sexes were injected with 2 mls 1% glycogen (from shellfish) as a chemotactic agent for stimulation of peritoneal exudates (CHAMBERS & GRAND 1936). The peritoneal macrophages were harvested by a wash-out technique (CONNING & FIRTH 1969) and resuspended in medium 199 (Biocult Labs) containing 20 IU heparin/ml to yield a concentration of approximately 10^6 cells/ml. Conical centrifuge tubes (115 x 25 mm); treated with a silicone water repellant to retard macrophage adhesion, were used as culture vessels. Cultures were stirred with a 12 mm PTFE coated bar magnet rotated at 180-200 rpm on a magnetic stirrer and maintained at 37° in a constant temperature room. Particulate suspensions in physiological saline were added to give a final dust concentration of 0.5 mg/10^6 cells. After two hours, 1 ml of culture was mixed with an equal volume of 0.5% trypan blue in phosphate buffered saline pH 7.4 (PAPPENHEIMER 1917) and a sample of the mixture examined with a x40 phase contrast objective. Random fields were examined for live and dead cells containing dust visible by optical microscopy and in addition for live and dead cells without visible dust. Where soluble materials were examined an aliquot was added from a stock solution to give the desired final concentration in the medium. Subsequent examination for live and dead cells was as previously described.

of a non-cytotoxic PVC powder (D) and of another non-cytotoxic polymer (polymethyl methacrylate) were stirred with 5 mls of 10% alkyl benzene sulphonate in alcoholic suspension for 30 minutes, recovered by centrifugation and washed four times with 25 ml aliquots of saline before resuspension as a stock solution. Results of these tests are shown in Table 5 from which it is obvious that surfactant treatment resulted in a significant increase in cytotoxicity of the particles. Thus it may be tentatively concluded that surfactant residues are responsible for the observed cytotoxicity of emulsion type PVC powders.

The original test of Styles & Wilson suspended the macrophages in 5% calf serum. The serum has been omitted from this series of tests as it has been observed (Styles & Pigott, unpublished observations) that such omission expands the observed range of cell mortality with no apparent effect on the relative toxicity of a variety of dust samples. Although the test in either form has been found unreliable where a soluble component is present in the particles, a series of tests with serum present was performed, as this may be more relevant to human exposure. Results are given in Table 6. It may be seen that non only was there a marked reduction in cell mortality but also a small change in relative toxicity with respect to α quartz. A parallel reduction in cell death with surfactant alone was also observed, reinforcing the belief that this agent is responsible for observed cytotoxicity.

CONCLUSIONS

The test of Styles & Wilson has previously shown a good correlation with fibrogenicity in-vivo for a wide range of powders, both organic and inorganic, provided no soluble component is present in the particles. On the basis of this test the PVC samples examined would not be expected to produce lung fibrosis. The cytotoxicity associated with the emulsion type polymers seems to be associated with the presence of surfactant residues and is reduced, but not abolished by the presence of serum in the culture medium. The relevance of this to an in-vivo situation is doubtful as it is well established that the in-vitro cytotoxic effects of surfactants generally are not necessarily indicative of high toxicity in-vivo. In any event the use of in-vitro testing for polymers with diffusible additives may not be relevant to longterm tissue reactions (STYLES 1974).

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TABLE 3 - Alcohol Wash Powders

Sample	% cells with dust	% dust bearing cells, dead
F	74	10.5%
G	61	14.9%

TABLE 4 - Survival of Macrophages in Surfactant Solution

Conc^{n} of Surfactant (w/v)	% cell death	
	Test	Control
0.01%	100%	5%
0.005%	100%	8%
0.002%	88%	4%
0.001%	76%	8%
0.0005%	63%	8%
0.0002%	12%	10%

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6.6. *In Vivo* Skin Capillary Abnormalities in Vinyl Chloride Workers

H. R. MARICQ, M. N. JOHNSON, C. L. WHETSTONE, and E. C. LeROY

Some workers exposed to vinyl chloride (VC) have been found to have clinically detectable proliferative and fibrotic lesions of the periphery (acroosteolysis, Raynaud's phenomenon, and sclerodermalike skin plaques) and the viscera (angiosarcoma and hepatic fibrosis, Banti type) (Harris and Adams, 1967; Wilson *et al.*, 1967; Dinman *et al.*, 1971; Jühe and Lange, 1972, Falk *et al.*, 1974; Lange *et al.*, 1974; Marstellar *et al.*, 1975; Thomas *et al.*, 1975).

Techniques of wide-field capillary microscopy have shown distinctively abnormal patterns in patients with spontaneous Raynaud's syndrome and scleroderma, as well as a strong positive correlation between severity of capillary pattern abnormality and visceral involvement (Maricq and LeRoy, 1973; Maricq *et al.*, 1974). Therefore, these techniques were used to examine 152 VC workers (44 selected, 108 unselected) and 50 control manual workers not exposed to VC.

In 44 VC workers selected for known disease (angiosarcoma, hepatic fibrosis, acroosteolysis, Raynaud's phenomenon, and skin lesions), 39% had capillary abnormalities, of which 16% were sclerodermalike patterns and 23% were isolated, discrete capillary lesions.

The possibility that trauma and not VC was the cause of capillary abnormalities in VC workers, many of whom had been reactor cleaners, was studied by comparing 108 VC workers and 50 manual workers outside the VC industry.

The results showed (Table I) that capillary abnormalities were significantly more common in the VC-exposed group than in non-VC manual workers ($\chi^2 = 15.50$, df = 2, $p < 0.001$). More of the selected VC workers with VC-specific lesions (angiosarcoma, hepatic fibrosis, severe acroosteolysis, and fibrotic skin changes) had capillary abnormalities than did VC workers with inactive, stable, or nonspecific lesions (healed acroosteolysis, nonspecific skin rashes, and Raynaud's phenomenon alone) ($\chi^2 = 13.85$, df = 1, $p < 0.001$). Among the 108 VC employees, no correlation was found between capillary abnormalities and length of employment or length of reactor-cleaning assignment.

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September 16, 1981

Fran Lichtenberg
Society of the Plastics Industry
355 Lexington Ave.
New York, N.Y. 10017

Dear Fran:

Enclosed is some material that I spoke to you about on residual VC in PVC and ambient exposure levels.

I hope it will be of some use to you and if you have any questions or need additional information, please contact me.

There is no need to return this material.

Cordially,

M. N. Johnson, M.D.
Director of Toxicology

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A Model for the Diffusion of Vinyl Chloride Monomer from PVC Under Various Conditions of Storage

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This example of employee safety activity in the vinyl resin industry is illustrative of the efforts put forth by many to achieve acceptable VCM levels in work areas during all conditions of handling. Since use conditions differ greatly at temperatures above T_g temperatures and are nonstatic, it was deemed necessary to develop a model embracing many variables to allow prediction of atmospheric vinyl chloride monomer levels for any combination of the variables.

INTRODUCTION

Within the past several years significant advancements have been made within the PVC industry to minimize employee exposure to vinyl chloride monomer (VCM). Improvements in PVC manufacturing processes to reduce the residual vinyl chloride monomer (RVCM) content of finished resins has been a key factor in maintaining acceptable VCM levels in work areas during packaging, processing, shipment and storage.

The diffusion of vinyl chloride monomer out of finished PVC resins has been adequately studied (*1*) at temperatures above the glass transition temperature (T_g). The results of these studies have been particularly useful in reducing RVCM during manufacturing and to predicting RVCM escape to the environment during processing. This new knowledge has also led to a rapid, simple gas chromatographic method (*2*) for the determination of RVCM in PVC from the analysis of the vapor phase (head space) over PVC powders in a closed container.

This somewhat ideal behavior does not exist at temperatures below T_g, however, and consequently one cannot use existing equilibrium data to predict the concentration of VCM in the air space above PVC resins and in particular under non-static conditions such as exists during the storage and transportation of bagged or bulk resin. In addition to the relative slow diffusion rate at or near ambient temperatures, other factors that affect VCM release include variable ventilation rates, mass-to-volume ratios, RVCM content of the resin and residence time in the storage or shipment compartment.

The primary objective of the research reported in this paper was to develop a physical model for the diffusion of VCM from PVC resin under various conditions of exposure during storage and shipment. The developed model is based on a statistically significant number of large scale diffusion experiments to show the interaction of all the above variables and to allow the prediction of atmospheric vinyl chloride monomer (AVCM) levels for any combination of the variables.

EXPERIMENTAL

It can be postulated that some reasonable understanding of these storage variables and their interactions can be developed from a physical modeling of a storage or shipment compartment. It can also be rationalized that the accuracy of such a model will be much improved if the experimental design is on a large scale that can physically simulate all of the storage variables but under highly controlled conditions. Any experimental design of this type, however, must be thoroughly tested to establish the validity of the test measurements and to assure the absence of any errors as may occur through poor test controls, adsorption of VCM by the system, and possible interfering components that could lead to spurious results.

Using a Computer Optimized Experimental Design (COED), 24 large scale experiments were selected to study the variables listed below to describe their effects and interaction on AVCM in a storage compartment or warehouse. The variables and ranges included:

1. Temperature—74-115°F
2. Ventilation rate—0.5-3.0 turnovers/h
3. Loading (PVC volume/storage volume)—11-34 percent
4. Resin RVCM—0.01-123 ppm
5. Storage time—1-170 h

In order to meet the demands of the statistically designed (COED) diffusion study, the experimental phase of this program had to be capable of controlling the following experimental parameters: temperature, ventilation rate, and mass/volume ratio. The basic physical model that was chosen to meet these requirements was one based on sealed metal storage bins (55-gal drums) housed in an environmental chamber where the temperature could be varied and controlled. A schematic of the entire experiment is shown in *Fig. 1*. The schematic