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LIFESPAN ORAL TOXICITY STUDY OF VINYL CHLORIDE IN RATS*

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Abstract—A lifespan oral toxicity study of vinyl chloride monomer (VCM) was carried out in Wistar rats, using five groups each of 60–80 males and 60–80 females. VCM was administered by incorporating polyvinyl chloride (PVC) powder with a high VCM content into the diet or by gastric intubation of a 10% VCM solution in soya-bean oil. The VCM doses (actual exposures) were 0 (control), 1·7, 5·0 and 14·1 mg/kg body weight/day provided by diets containing PVC powder, and 300 mg/kg body weight given by stomach tube as a solution of VCM in oil on 5 days/wk. The death rate was higher in all VCM-treated groups than in the controls and increased with increasing VCM doses. The 14·1- and 300-mg/kg treatments were associated with shortened blood-clotting times, slightly increased α-foetoprotein levels in the blood serum, liver enlargement and an increased haematopoietic activity in the spleen. A variety of neoplastic and non-neoplastic treatment-related liver lesions was found at each of the VCM levels. The changes varied from swollen and irregularly-shaped mitochondria in hepatocytes to hepatocellular carcinomas and hepatic angiosarcomas. The tumour response of the liver appeared to shift from a predominance of angiosarcomas at the highest dose level *via* a mixture of angiosarcomas and hepatocellular tumours at the intermediate levels to the exclusive development of hepatocellular tumours at the lowest VCM level. Tumours attributable to VCM exposure and found at other sites included pulmonary angiosarcomas, extrahepatic abdominal angiosarcomas and tumours of the Zymbal glands; these neoplasms occurred at VCM levels of 5·0 mg/kg and above. In addition, there was some evidence that VCM exposure enhanced the development of abdominal mesotheliomas and of adenocarcinomas of the mammary glands. Thus, the present study showed that orally administered VCM is a carcinogen in rats, and that the "no-observed-adverse-effect level" in rats was lower than 1·7 mg/kg body weight/day under the rigorous conditions of continuous oral VCM exposure resulting from the release of VCM from PVC powder present in the gastro-intestinal tract.

INTRODUCTION

Industrial exposure to vinyl chloride monomer (VCM) has been associated with disorders such as acro-osteolysis, non-malignant liver disease, angiosarcoma and carcinoma of the liver, and tumours of the brain and lungs and of the lymphatic and haematopoietic systems (Haley, 1975; Hopkins, 1980; Monson, Peters & Johnson, 1974; Rawls, 1980; Thomas & Popper, 1975; Vale, Kipling & Walker, 1976; Waxweiler, Stringer, Wagoner, Jones, Falk & Carter, 1976). In addition, various types of tumours, such as hepatic and extrahepatic angiosarcomas, hepatocellular tumours, mammary-gland carcinomas, Zymbal-gland tumours, nephroblastomas, brain tumours, pulmonary adenomas and nasal olfactory carcinomas, as well as a series of non-neoplastic lesions in several organs, have been found in a number of animal species after prolonged exposure to atmospheres containing VCM at sufficiently high concentrations (Basaliev, Vazin & Kotchetkov, 1972; Feron & Kroes, 1979; Lee, Bhandari, Winston, House, Dixon & Woods, 1978; Maltoni & Lefemine, 1975; Suzuki, 1978; Torkelson, Oyen & Rowe, 1961; Williamson, 1976; Winell, Holmberg & Kronevi, 1976).

Residual VCM present in extruded polyvinyl chloride (PVC) has been shown to be liable to migrate into PVC-packed foods and drinks (Daniels & Proctor, 1975; Fuchs, Gawell, Albanus & Slorach, 1975; Potter, 1976; Randolph, 1973; Williams & Miles, 1975). There is still only a small amount of data on the oral toxicity of VCM. In a 13-wk toxicity study, in which the monomer was dissolved in soya-bean oil and administered to rats at levels of 0, 30, 100 or 300 mg/kg body weight, once daily on 6 days/wk, the no-effect-level was conservatively placed at 30 mg/kg body weight, but was probably higher since the effects at the higher doses were of doubtful toxicological significance (Feron, Speek, Wiliems, van Battum & de Groot, 1975). From preliminary observations it appeared that a more practical method for chronic oral exposure of rats to VCM is the feeding of diets containing PVC powder with a high VCM content (Feron *et al.* 1975). Therefore, in the present lifespan study, PVC powder containing VCM was incorporated in the diet at levels to give planned daily intakes of 1, 3 or 10 mg VCM/kg body weight. This method does not allow dietary VCM intakes much higher than 10 mg/kg body weight/day, a dose that is, however, very high in comparison with a recent estimate of the maximum likely oral daily intake by man, i.e. 0·0017 μg VCM/kg body weight/day or 0·1 μg VCM/

*The study was sponsored by a group of co-operating European industrial concerns, including Verband Kunststofferzeugende Industrie e.V. (FRG), Shell Nederland Chemie, Dutch State Mines, Akzo Zout Chemie Nederland B.V. and Dow Chemical Europe S.A.

man/day (Ministry of Agriculture, Fisheries and Food, 1978). On the other hand, 10 mg VCM/kg body weight/day is low when compared with the doses used in the aforementioned 13-wk study (Feron *et al.* 1975). Since a chronic toxicity study should include at least one effect-producing level, a group of rats given VCM in soya-bean oil by gavage at a level as high as 300 mg/kg body weight/day (on 5 days/wk) was included in the present experiment, despite the disadvantages of gastric intubation.

EXPERIMENTAL

Test materials. Vinyl chloride monomer (VCM) was obtained from Akzo Zout Chemie, Rotterdam, in pressurized stainless-steel cylinders. The product had the following standard specification: vinyl chloride monomer $\geqslant 99{\cdot}97\%$ w/w; acetylene $\leqslant 2$, monovinylacetylene $\leqslant 15$, 1,3-butadiene $\leqslant 10$, methyl chloride $\leqslant 75$, ethyl chloride $\leqslant 50$, chloroprene $\leqslant 1$, 1,1-dichloroethane $\leqslant 1$ and 1,2-dichloroethane $\leqslant 20\ \mu$l/litre (gas); acetaldehyde $\leqslant 5$, hydrochloric acid $\leqslant 1$, iron $\leqslant 0{\cdot}5$, water $\leqslant 100$ and evaporation residue $\leqslant 10$ mg/kg.

PVC powder (Carina S 65-02) was supplied by Shell Nederland Chemie, Pernis. The particle-size distribution (by weight), was max $0{\cdot}1\%$ > 300, max 4% > 200, max 90% > 88 and max 95% > 40 μm. The VCM content of a portion of the PVC powder was raised to approximately 4000 ppm by mixing the powder with a calculated amount of liquid VCM in a closed steel barrel. This PVC powder was stored in tightly closed steel containers in a refrigerator at 4°C until a few minutes before being mixed with the diet. Part of the PVC powder obtained from Shell was freed from VCM by being kept in thin layers in a vacuum oven at 60°C for a period of 3–4 days. After this treatment the VCM content of the PVC powder was found to be less than 0·3 ppm.

A 10% solution of VCM in soya-bean oil was prepared by injecting liquid VCM into the oil. The VCM concentration was checked by gas–liquid chromatography according to the method described previously (Feron *et al.* 1975). The solution was stored at 4°C for a maximum period of 4 wk.

Preparation and administration of PVC-containing diets. Each diet contained 10% PVC powder, with varying proportions of VCM-containing and 'VCM-free' (i.e. <0·3 ppm VCM) powders. Thus the control diet contained 10% VCM-free PVC powder and the high-dose diet 10% PVC powder containing 4000 ppm VCM, while the diets for the low- and mid-dose groups contained, respectively, 1 and 3% VCM-containing PVC powder with the balance of 9 and 7% PVC made up with powder without VCM. The various diets were prepared daily by mixing appropriate amounts of PVC powder (with or without VCM) with the Institute's rat stock diet just prior to their being offered to the rats. The diets were available to the rats each day for a period of four consecutive hours (generally between 09.00 and 15.00 hr); thereafter, the rats had no food until the 4-hr feeding period on the next day. The rats had constant access to bottled, unfluoridated tap-water.

Calculation of the oral VCM exposure levels. To calculate the actual oral exposure levels of VCM provided by VCM-containing PVC powder in the diets, the following information was needed: (a) the amount of VCM evaporating from the diets during the 4-hr feeding period; (b) the speed with which the animals consumed the food during this period; and (c) the amount of VCM excreted in the faeces.

The rate of evaporation of VCM from the diets was determined by measuring the VCM content of the diets at the beginning of the feeding period and after 1, 2 and 4 hr. Samples to be analysed were taken at random from the feeders in the cages, without homogenization of the diets in the feeders. Each test diet was sampled in this way on 11 or 12 different days and analysed for VCM content by gas–liquid chromatography (Feron *et al.* 1975). The average VCM contents of the various diets at the different times are depicted in Fig. 1.

The eating-speed was determined by measuring the amount of residual feed in the feeders after periods of 1, 2 and 4 hr. Since no appreciable variations were encountered in the rate of food consumption by the animals in the various groups, average rates of food consumption were calculated for males and for females (Fig. 1).

The VCM intake (mg/kg body weight/day) by male and female rats of each group was calculated from the graphs representing the rate of evaporation of VCM from the diets (Fig. 1) and the rate of food consumption over the 4-hr feeding period. Both graphs were

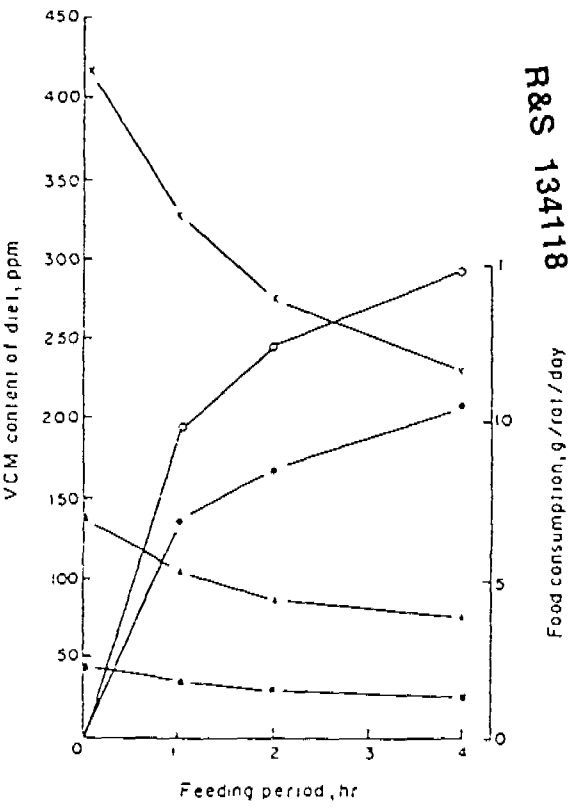

Fig. 1. Average VCM levels of the high-dose (×), mid-dose (▲) and low-dose (■) diets and average amounts of food consumed by males (O) and females (□) at different times during the 4-hr feeding period

assumed to consist of three straight lines, one line for the first hour of the feeding period, one for the second hour and one for the last two hours. The VCM intake during each of these three periods was calculated by multiplying the amount of food eaten during a certain period with the average VCM content of the food in that period. The total VCM intake, the sum of the VCM intakes during these three periods, was subsequently expressed as a percentage of the theoretical intake, calculated from the average total amount of food consumed and the VCM content of the diet at the initiation of the feeding period. This figure was found to be 82·4, 79·1 and 79·1% for males, and 81·3, 79·3 and 79·0% for females of the low-, mid- and high-dose groups, respectively. The overall average for males and females of the various test groups was calculated to be 80%.

To measure faecal excretion of VCM, freshly produced faeces from a representative number of rats of each test group were collected at 09.00 hr (1 hr before the start of the feeding period), at 14.00 hr (at the end of the feeding period) and at 18.00 and 23.00 hr (4 and 9 hr after termination of feeding). Fresh faecal samples were obtained by squeezing the lower part of the rat's abdomen. The droppings were weighed, submerged in 10 ml ethyl acetate, and stored at 4°C in a closed vessel prior to analysis of the supernatant liquid by gas chromatography. Within a particular test group there were no appreciable differences in the VCM levels of faeces collected at the various times, although the optimum value was invariably obtained from faeces collected 9 hr after termination of feeding (at 23.00 hr). The average amount of VCM found in the faeces, expressed as a percentage of the VCM intake, was found to be 8, 10 and 17% for the low-, mid- and high-dose groups, respectively.

The VCM content of freshly prepared test diets was determined regularly, some twenty determinations for each dosage level being carried out during the study. Daily determinations were not considered necessary because the VCM content of the PVC powder stored at 4 C in a closed barrel was found to remain constant, and the VCM content of the PVC powder used appeared to correspond very well with the VCM content of the various diets. From these VCM determinations, the average VCM contents of the various diets were calculated as 46, 139 and 424 ppm for the low-, mid- and high-dose groups, respectively. Since both the loss of VCM from the diets before consumption and the VCM content of the faeces were known, the actual oral exposure levels of VCM could be calculated. They were found to be 1·7, 5·0 and 14·1 mg/kg body weight/day for the low-, mid- and high-dose groups (Table 1).

VCM in oil administered by gavage. One group of rats received VCM in oil by gavage. The approximate dose was 300 mg/kg body weight, administered daily on 5 days/wk for 83 wk, in calculated volumes of a 10% VCM solution in soya-bean oil. The volumes were adapted to the mean body weights once every week if necessary. These rats were offered the Institute's stock diet for rats and bottled unfluoridated tap-water *ad lib.*

Animals and housing. Newly weaned albino Wistar rats (Cpb: WU; Wistar random), obtained from the SPF colony of the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, were allocated randomly over five groups of males and five groups of females in such a way that the mean body weights were virtually the same. The control group and the two highest dosage groups each consisted of 80 males and 80 females. The two lowest dosage groups each consisted of 60 males and 60 females. During the 5 days before the start of the experiment, the rats that were to be fed the PVC-containing diets received stock diet without PVC for 4–6 hr each day, to adapt them to the daily feeding period of 4 hr. At the start of the study the rats were 5 wk old. The study was terminated when about 75% of the control rats were dead, a point reached for males in wk 135 and for females in wk 144.

The rats receiving PVC powder in their diet were housed under conventional conditions, in groups of five in suspended stainless-steel cages with a wire-screen bottom in a well-ventilated room maintained at 24 ± 1°C. The rats given VCM in oil by gavage were housed in groups of two in suspended, tinned wire-screen cages in a well-ventilated cabinet maintained at 25–28°C. Animals in bad condition were housed individually in separate cages until they died or were killed *in extremis.*

Conduct of the experiment. The rats were individually weighed, initially, at wk 1, 2, 4, 6, 8, 10 and 12, and at 4-wk intervals thereafter. Food consumption of

Table 1. *Designed and actual dosage of VCM in rats maintained on diets containing PVC powder*

Designed VCM treatment			Oral intake of VCM (mg/kg body weight/day)		Actual oral exposure level of VCM‡ (mg/kg body weight/day)
Dietary level (ppm)	Intake (mg/kg body weight/day)	Actual initial dietary VCM level (ppm)*	Theoretical†	Actual	
0	0	0	0	0	0
20	1	46	2·3	1·8	1·7
60	3	139	7·0	5·6	5·0
200	10	424	21·2	17·0	14·1

*Average dietary VCM contents determined immediately after preparation of the diets.

†Assuming no loss of VCM by evaporation from the diets (see Fig. 1).

‡Oral intake of VCM diminished by the faecal VCM, which was found to be 8, 10 and 17% of the actual oral VCM intake for the low-, mid- and high-dose groups, respectively. The VCM excreted in the faeces was considered to be still enclosed in the PVC granules and thus not to have been in contact with the body.

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20 rats/sex/group was measured during wk 1–4, 10–11, 24–25, 36–37, 60–61, 72–73 and 84–85.

Blood samples were collected from the tip of the tail of ten rats/sex/group in wk 13, 26, 52, 78 and 94. All samples were used for determinations of haemoglobin concentration (cyanmethaemoglobin method of van Kampen & Zijlstra, 1961) and packed cell volume (microhaematocrit), and for thrombocyte and red and white blood cell counts (Coulter Counter), and differential white cell counts by direct visual count of smears after Pappenheim staining (Gorter & De Graaff, 1955). Fasting blood glucose (Technicon AutoAnalyzer method N-9a) and blood-urea nitrogen (Ceriotti & Spandrio, 1965) were determined in wk 13, 26, 52 and 106. The analyses were conducted upon blood from the tip of the tail of ten rats/sex/group after the animals had been fasted overnight. During wk 13, 26, 52 and 106, samples of blood were collected from the orbital sinus of ten rats/sex/group. The following measurements were made in the serum after centrifugation at 3000 rpm for 20 min: alkaline phosphatase (Bessey, Lowry & Brock, 1946), glutamic–oxalacetic transaminase and glutamic–pyruvic transaminase (Reitman & Frankel, 1957), total protein (biuret reaction), albumin (De Leeuw-Israël, Arp-Neefjes & Hollander, 1967) and serum-protein pattern (Wieme, 1965).

Individual urine samples, collected from ten rats/sex/group in wk 13, 26, 52, 78 and 94, in each case during the last 16 hr of a 24-hr period of deprivation of food and water, were measured for volume (in calibrated tubes), specific gravity (by an Abbe-type refractometer), uric acid (Gorter & De Graaff, 1955) and glutamic–oxalacetic transaminase activity (Reitman & Frankel, 1957). Semi-quantitative measurements were made of pH, protein, sugar, occult blood and ketones in pooled urine samples, using Labstix from Ames Laboratories. Deposits obtained by centrifugation at 3000 rpm for 3 min were examined microscopically for erythrocytes, leucocytes, epithelial cells, amorphous substances, phosphate crystals, casts, bacteria, worm eggs and sperm cells.

All males still alive in wk 135 and all females surviving to wk 144 were killed by decapitation, autopsied and subjected to a careful gross examination. A thorough autopsy was also performed on rats found dead or killed *in extremis*. Samples of the following organs were fixed in 10% neutral formalin: heart, kidneys, liver, spleen, brain, testes, ovaries, pituitary, thyroid, adrenals, thymus, pancreas, epididymides, prostate, coagulating glands, seminal vesicles, preputial glands, mammary glands, lungs, skeletal muscle, spinal cord, sciatic nerve, urinary bladder, parotid, sublingual and submaxillary salivary glands, axillary and mesenteric lymph nodes, nose, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, skin, femur with joint and bone marrow, trachea, aorta, exorbital lachrymal glands, Zymbal glands, cervix and uterus. The organs to be examined microscopically were processed through paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin. Microscopic examination of all organs preserved was carried out in 20 males and 20 females of the control group and of each of the two highest dosage groups. For the control groups these 20 males and 20 females comprised all animals killed at the end of the experimental period, supplemented by the animals that had lived longest before they had to be killed in a moribund condition. In the two highest dosage groups the rats subjected to detailed histopathology comprised the 20 males and 20 females that had lived longest before being killed in a moribund condition. Histopathological examination of all other rats was restricted to the liver, Zymbal glands, lungs, kidneys, spleen, pituitary, thyroid, adrenals, grossly visible tumours and organs containing gross lesions suspected of being tumours.

Batches of ten males and ten females from the control group and the two highest dosage groups were killed by decapitation and subjected to a thorough autopsy after 26 and 52 wk (interim kills). At these times, the following were recorded: blood-clotting time (Normotest reagents from Nyogaard and Co., Oslo, Norway), serum electrolytes Na, K, Ca, Mg (Paschen & Fuchs, 1971) and Cl (coulometric method), serum alkaline phosphatase, serum glutamic–oxalacetic transaminase, serum glutamic–pyruvic transaminase, total serum protein, albumin and serum-protein patterns (determined by the methods mentioned before), lactic dehydrogenase (Wróblewski & LaDue, 1955), serum α-foetoprotein* (Boekestein-Tjahjadi & Kroes, 1976), liver function (bromosulphophthalein-extinction test and barbiturate sleeping-time method), kidney function (phenol-red excretion test: Sharratt & Frazer, 1963), activities of aminopyrine demethylase (Gram, Wilson & Fouts, 1968) and aniline hydroxylase (Gilbert & Golberg, 1965) in liver preparations, and weights of liver and kidneys. Histopathology of the liver, kidneys and Zymbal glands was studied and the liver was also examined by light microscopy of semi-thin Paragon-stained plastic sections and by electron microscopy. For the latter the liver was fixed by perfusion with 1·5% glutaraldehyde buffered with 0·067 M-sodium cacodylate containing 1% sucrose (pH 7·4) at 4°C for 17 hr and this was followed by post-fixation in cacodylate-buffered 1% OsO_4, dehydration in graded acetone–water mixtures, and embedding in Epon 812.

Additional control group. One extra control group of 60 male and 60 female rats was housed in a room separate from that used for the other rats fed PVC-containing diets, thus preventing any possible contact of these control rats with VCM by inhalation. The diet containing 10% PVC containing no VCM was fed to the animals in this group *ad lib.* instead of for the restricted daily feeding period of 4 hr used for the real control group. Body weights and mortality were recorded, and all the animals were autopsied. The organs and tissues were preserved, but no slides were prepared.

RESULTS

Behaviour and appearance

During yr 2 of the test the general condition of the rats in the 300-mg/kg group gradually declined and administration of VCM by gavage became increasingly difficult. Many rats grew lethargic and filthy

*The α-foetoprotein determinations were carried out in the Laboratory of Pathology of the National Institute of Public Health, Bilthoven, under the supervision of Dr R. Kroes.

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before they died or were killed in a moribund condition. Since most of these rats had severe lesions, including tumours, of the liver and lungs, the VCM treatment of this group was discontinued at wk 84.

After month 18 the number of unthrifty rats in the 5·0 and 14·1-mg/kg groups gradually increased, more rapidly in the latter (high-dose) group and more rapidly in females than in males. The poor condition started with a humpbacked position and slight emaciation followed by pale eyes, lethargy, filthiness and often severe emaciation. Liver masses could be detected in many of these rats by abdominal palpation. Swollen abdomens were occasionally seen, generally associated with multiple intra-abdominal tissue masses detectable by abdominal palpation. In addition, animals with breathing difficulties were fairly common in these groups; their lungs were invariably found to contain multiple nodules, which appeared microscopically to be primary angiosarcomas or metastases from either hepatic angiosarcomas or hepatocellular carcinomas. External tissue masses occurred fairly often, mainly after month 18; most appeared to be mammary-gland tumours, but there were also other types of tumours originating from the skin, subcutis or dermal adnexa.

Randomly distributed major abnormalities, not attributable to VCM, included staring coats, a bloody discharge from the nose, wet stools, focal alopecia, focal dermatitis, blood around the muzzle and eyes, paresis of the hind legs, loss of one or both eyes, and a white opaque cornea.

Body weights and food consumption

Body weights (Fig. 2) and food intakes of the rats fed the VCM-treated diets were very similar to those of the controls. The rats of the additional control group were much heavier than those of the other groups receiving diets containing PVC powder. This difference was undoubtedly due to the fact that the extra controls had constant access to their food, whereas diet was available to the other rats only during a period of four consecutive hours each day. The rats of the 300-mg/kg group had constant access to stock diet and had much higher body weights than the animals fed PVC-containing diets for a limited period each day, but their body weights were lower than those of the additional control group.

Mortality

Mortality was low in males and females of the 4-hr-fed control group during the first 2 yr of the test, amounting to 10% after 2 yr (Table 2). At this time the mortality among rats of the additional control group was some three times higher (about 30%).

About 40% of the males and females of the 300-mg/kg group had already died by month 18 of treatment. Thereafter, mortality rapidly increased, and by just after month 24 all the animals in this group had died, mainly from pulmonary or hepatic insufficiency resulting from neoplastic or non-neoplastic lesions in these organs. A striking and dose-related increase in death-rate was also found in the 5·0- and 14·1-mg/kg groups, with females dying earlier than males. In the low-dose group (1·7 mg VCM/kg body weight), the mortality of males was comparable to that of the male controls, and the death rate of females was only slightly higher than that of female controls.

Haematology, biochemistry, urine analysis and organ function

No relevant changes were found in any of the parameters studied, except for a shorter blood-clotting time and an increased content of α-foetoprotein in the blood serum of animals in the group fed the highest VCM dose and in the intubated group (Table 3).

Gross pathology

Liver-to-body weight ratios were higher in the 14·1- and 300-mg/kg groups than in the control group after both 26 and 52 wk (Table 3). Many rats exposed to VCM had severe liver lesions. Pronounced swelling, discoloration and altered consistency of one or more lobes, often with varying numbers of cysts, were common findings, as were nodules and nodule-like processes, varying widely in size (up to 4 cm in diameter), appearance and consistency. Many of the nodules were solid and pale; others were cystic and haemorrhagic. The larger firm and pale nodules with central necrosis appeared, upon microscopy, to be carcinomas. These were found mainly in completely distorted livers. Angiosarcomas were most often seen as multiple soft dark cystic nodules, containing blood and granular necrotic material. Nodules, which were later classified as 'neoplastic nodules', were relatively small, firm and compact; they were either pale or had the same colour as the adjacent normal liver tissue, and never contained necrotic material. These liver changes were most pronounced and occurred earliest

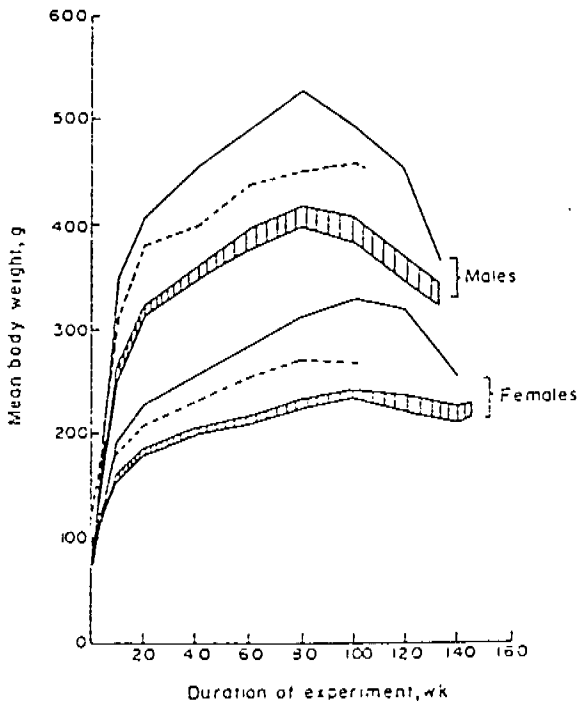

Fig. 2. Average body weights of the extra controls fed the 10%-PVC diet *ad lib* (—) and of the rats given 300 mg VCM kg body weight in oil by gavage (– – –). The weight curves of the rats receiving 0, 1·7, 5·0 or 14·1 mg VCM/kg body weight/day from the 10%-PVC diets fed for 4 hr each day all lie within the shaded area.

Table 2. *Cumulative mortality of rats exposed orally to VCM for up to 2·7 yr*

Treatment group (mg VCM/kg body weight/day)	Number of deaths† by end of wk:									
	12	36	52	80	92	105	120	128	134‡	143‡
Males										
0	0	0	0	0	2	6	18	40	46	
1·7	0	0	1	1	3	6	13	37	40	
5·0	0	0	0	2	7	12	30*	49	60**	
14·1	0	1	2	8**	22***	40***	56***	60***	60**	
300§	0	6	6	23	47	53	60	60	60	
0·	0	0	0	1	3	19	28	46	46	
Females										
0	0	0	0	1	5	6	22	27	32	41
1·7	0	0	1	2	4	13	26	32	34	55**
5·0	0	1	2	7*	16**	31***	55***	60***	60***	60***
14·1	0	0	1	7*	43***	60***	60***	60***	60***	60***
300§	0	3	7	24	47	58	60	60	60	60
0‡	0	0	1	4	10	17	27	42	43	52

†Initial number of rats: 60/sex/group.
‡Surviving males were killed in wk 135 and surviving females in wk 144.
§The figures for this group were not evaluated statistically, because no corresponding control group was included in the study.
·Additional control group housed in a separate room and having constant access to the diet containing 10% PVC without VCM.
Values marked with asterisks differ significantly from those of the controls according to the chi-square test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

and most frequently in the 300- and 14·1-mg/kg groups. Angiosarcomatous nodules were not seen at all in the low-dose group.

Pulmonary alterations that could be ascribed to VCM treatment consisted of small haemorrhagic or greyish nodules, often located at the edge of a pulmonary lobe. These changes were not observed in controls or low-dose animals.

Rats with a swollen abdomen due to ascites and to numerous pale firm nodules (diameter 2–15 mm) in the peritoneum were encountered slightly more frequently in each of the three lowest dosage groups (maximum incidence 15%) than in the control or 300-mg/kg groups (maximum incidence 5%). Microscopically the nodules appeared to be mesotheliomas.

Many other types of gross changes, both neoplastic and non-neoplastic, were seen either frequently or just in a few animals, but there was no indication that any of these changes were related to VCM.

Light microscopy of the liver

The type and incidence of treatment-related histopathological changes found in the liver are given in Table 4. The incidence of foci of cellular alteration

Table 3. *Mean prothrombin times, α-foetoprotein contents of the blood serum, and liver weights of rats exposed orally to VCM for 26 or 52 wk*

Treatment group (mg VCM/kg body weight/day)	Prothrombin time (sec) at wk		α-Foetoprotein (μg/ml) at wk		Liver weight (g/100 g body weight) at wk	
	26	52	26	52	26	52
Males						
0	41·3	41·6	75	30	2·54	2·63
14·1	38·9*	38·8*	74	51*	2·91*	3·00
300†	35·2	37·3	84	55	3·31	3·38
Females						
0	37·2	32·8	99	53	2·60	2·57
14·1	34·1***	30·7	91	109**	3·08**	3·31*
300†	31·7	30·5	93	56	3·86	3·44

†The figures for this group were not evaluated statistically, because no corresponding control group was included in the study.
Values marked with asterisks differ significantly from those of the controls according to the test of Wilcoxon (prothrombin time and α-foetoprotein) or Student's *t* test (liver weight): $*P < 0.05$, $**P < 0.01$; $***P < 0.001$.

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Table 4. *Type and incidence of treatment-related histopathological changes in the liver of rats exposed orally to VCM*

Type of change†	Treatment group (mg VCM/kg/day)...	Incidence of change									
		Males					Females				
		0	1·7	5·0	14·1	300‡	0	1·7	5·0	14·1	300‡
Animals killed after 26 wk											
	No. of rats examined ...	10	§		10	9	10	—	—	10	10
Clear-cell foci		0	—	—	1	1	0	—	—	5**	2
Animals killed after 52 wk											
	No. of rats examined...	9	—	—	10	9	9	—	—	10	8
Clear-cell foci		1	—	—	8**	0	0	—	—	8**	0
Basophilic foci		0	—	—	0	0	0	—	—	4	1
Eosinophilic foci		0	—	—	2	0	0	—	—	5**	0
Neoplastic nodule		0	—	—	1	0	0	—	—	2	0
Hepatocellular carcinoma		0	—	—	1	0	0	—	—	1	0
Cystic proliferation of bile ducts		0			0	0	0	—	—	4*	0
Animals found dead or killed *in extremis* or terminally											
	No. of rats examined...	55	58	56	59	55	57	58	59	57	54
Clear-cell foci		0	9**	16***	21***	9	4	24***	22***	36***	10
Basophilic foci		8	18	21*	22**	12	0	33***	17	28***	19
Eosinophilic foci		3	23***	27***	33***	11	8	35***	20*	29***	6
Neoplastic nodule		0	1	7**	23***	3	2	26**	39***	44***	2
Hepatocellular carcinoma		0	1	2	8**	1	0	4	19***	29***	0
Angiosarcoma		0	0	6*	27***	27	0	0	2	9**	29
Proliferation of atypical sinusoidal cells only		2	0	4	7	6	4	6	3	4	7
Extensive necrosis		4	4	8	23***	21	5	6	19***	27***	24
Cysts		2	3	4	16***	3	9	30***	41***	49***	3
Liver-cell polymorphism		4	16*	28***	42***	36	34	51*	38	41	41
Centrilobular degeneration		0	0	0	1	1	1	2	3	1	18
Focal haematopoiesis		0	1	0	10**	8	1	3	1	6	12

†Specific hepatocellular lesions were classified according to Squire & Levitt (1975)

‡The figures of this group were not evaluated statistically, because no corresponding control group was included in the study.

§Not examined.

The initial number of animals was 60/sex/group. A number of rats could not be examined because of cannibalism or advanced autolysis.

Values marked with asterisks differ significantly from those of the controls according to the chi-square test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

was much higher in each of the three test groups receiving VCM-containing PVC powder (the 1·7-, 5·0- and 14·1-mg/kg groups) than in the control group, and in addition, was nearly always higher than in the group receiving VCM in oil by gavage (300-mg/kg group). Similar differences also existed for neoplastic nodules and hepatocellular carcinomas. In the test groups receiving VCM-containing diets, the incidence of both neoplastic nodules and hepatocellular carcinomas was positively related to the VCM dose, and was much higher in females than in males.

Angiosarcomas of the liver (Figs 3 and 4) were found in males and females of the three highest dosage groups, but did not occur at all in controls and low-dose animals (Table 4). In both the 5·0- and 14·1-mg/kg groups the incidence of angiosarcomas was three times higher in males than in females. This difference between the sexes did not exist in the 300-mg/kg group, in which an angiosarcoma incidence of about 50% was found in both males and females.

In the 300-mg/kg group, the average latent period for the detection of liver tumours (almost exclusively angiosarcomas) at death was found to be 84 wk for males and 83 wk for females. In the 14·1-mg/kg group these average latent periods appeared to be 104 and 88 wk for males and females, respectively, clearly indicating an earlier appearance of liver tumours in the former group especially in males.

Several rats bearing angiosarcomas or liver-cell tumours also showed focal proliferation of atypical sinusoidal cells, often accompanied by distension of sinusoids. In addition, similar changes were observed in several rats not bearing a tumour in the liver. Large areas of necrosis were found in the livers of a fairly large number of rats of the three highest dosage groups. Cysts, very probably lined by proliferated bile-duct epithelium, were seen in a relatively large number of males of the 14·1-mg/kg group, and in females of the 1·7-, 5·0- and 14·1-mg/kg groups. The size and multiplicity of the cysts varied widely in individual animals. Cellular and nuclear polymorphism of hepatocytes was much more common in the test groups than in controls. Centrilobular liver degeneration was a frequent finding in females, but not in males, of the 300-mg/kg group. Focal haematopoiesis was encountered more often in males and females in the 14·1- and 300-mg/kg groups than in those of the other groups.

Electron microscopy of hepatic parenchyma

In semi-thin sections, foci of hepatocytes and also isolated hepatocytes with a finely 'vacuolized' cytoplasm were found in rats of the 14·1- and 300-mg/kg groups after both 26 and 52 wk. Ultrastructurally the 'vacuoles' appeared to represent swollen mitochondria with a pale matrix and short cristae (Fig. 5). The abnormal mitochondria closely resembled those found in rats following inhalation exposure to 5000 ppm VCM for periods of 4–52 wk and already described by Feron, Spit, Immel & Kroes (1979b).

Foci of hepatocytes, each containing numerous highly swollen, irregularly-shaped mitochondria without cristae and a matrix widely varying in density, were encountered after 26 wk. An increased amount of tubular smooth endoplasmic reticulum (SER) was invariably present among the swollen mitochondria. Occasionally scattered individual hepatocytes were found to contain a few extremely swollen mitochondria but were otherwise normal, as were their other mitochondria. After 52 wk, large areas of hepatocytes containing numerous swollen mitochondria were found, mainly in rats of the 14·1-mg/kg group. These hepatocytes had large nuclei with pronounced nucleoli. Tubular SER and whorls of SER were occasionally found together with mitochondria containing lucent areas in which a membranous or flocky material was often encountered. Single membranes of rough endoplasmic reticulum surrounding these mitochondria had partially lost their ribosomes.

Light microscopy of organs other than the liver

A wide variety of alterations was found, nearly all apparently related to the normal ageing process. An exception may have been the very marked haematopoietic activity found in the spleen of six out of 40 males and ten out of 40 females from the two highest dosage groups, while in controls only slight to moderate splenic haematopoiesis was observed.

The site and type of tumours observed in organs other than the liver and their incidence in the different groups are presented in Table 5. Angiosarcomas were frequently found in the lungs at the two highest dose levels, and also occurred in a few rats of the 5·0-mg/kg group. They were most often seen as multiple small foci of tumour cells with an angiomatous growth pattern. Their appearance was highly suggestive of metastases. On the other hand, in several cases, the histological appearance of the neoplasms did not permit the exclusion of their being diagnosed as primary pulmonary angiosarcomas. In addition, in three rats with a pulmonary angiosarcoma, no angiosarcoma was encountered outside the lungs. In four rats from the 300- and 14·1-mg/kg groups an angiosarcoma was found in the abdominal cavity outside the liver, while the liver showed no signs of angiosarcoma formation. Therefore, these tumours were considered to be primary extrahepatic angiosarcomas.

Pulmonary metastases of hepatocellular carcinomas were not uncommon. A total of five tumours of the Zymbal glands (ceruminous glands) were found—two squamous-cell carcinomas in the 5·0-mg/kg group, and two squamous-cell carcinomas and one adenoma in the 300-mg/kg group. Abdominal mesotheliomas were observed in each of the groups, including the control group. In several groups their incidence was higher than in controls, but a positive dose–response relationship with respect to incidence was absent. On the other hand, the latent period for detection of abdominal mesothelioma at death was found to decrease with increasing dose levels for both males and females. The histological appearance of the peritoneal mesotheliomas varied, but two main types could be distinguished, a fibrous type in which sarcomatous areas predominated and an epithelial type consisting mainly of tubulo-papillary formations. In several tumours both the sarcomatous areas and the tubulo-papillary structures occurred to the same extent. Mitotic figures were never abundant.

Fibroadenomas of the mammary glands were much less frequent in females of the 5·0-, 14·1- and

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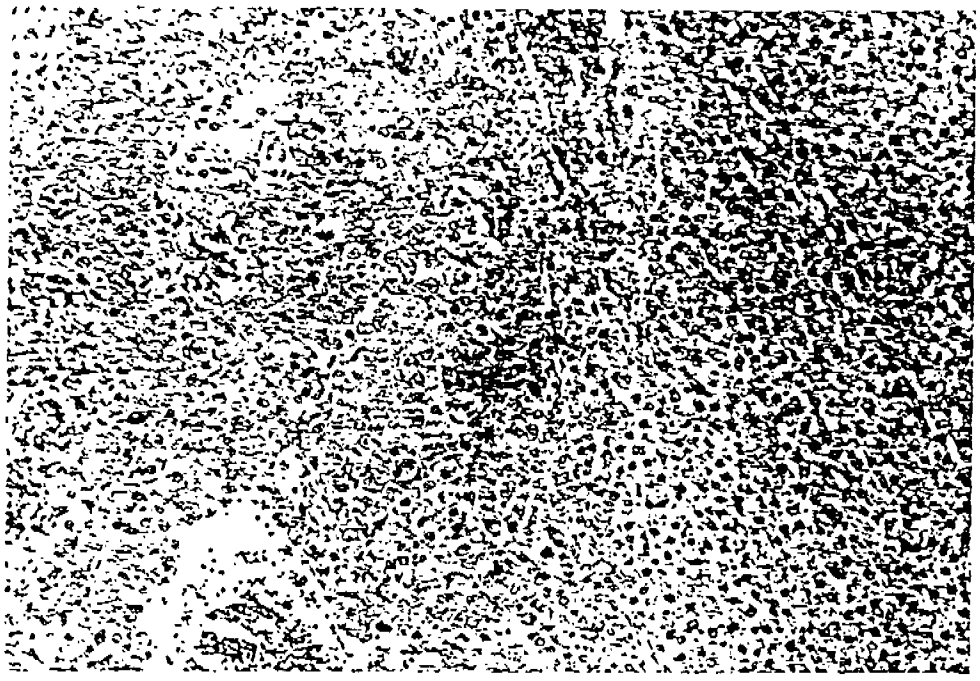

Fig. 3. Hepatic angiosarcoma of a male rat exposed orally to 14·1 mg VCM/kg body weight/day for a period of 101 wk. Haematoxylin and eosin ×160.

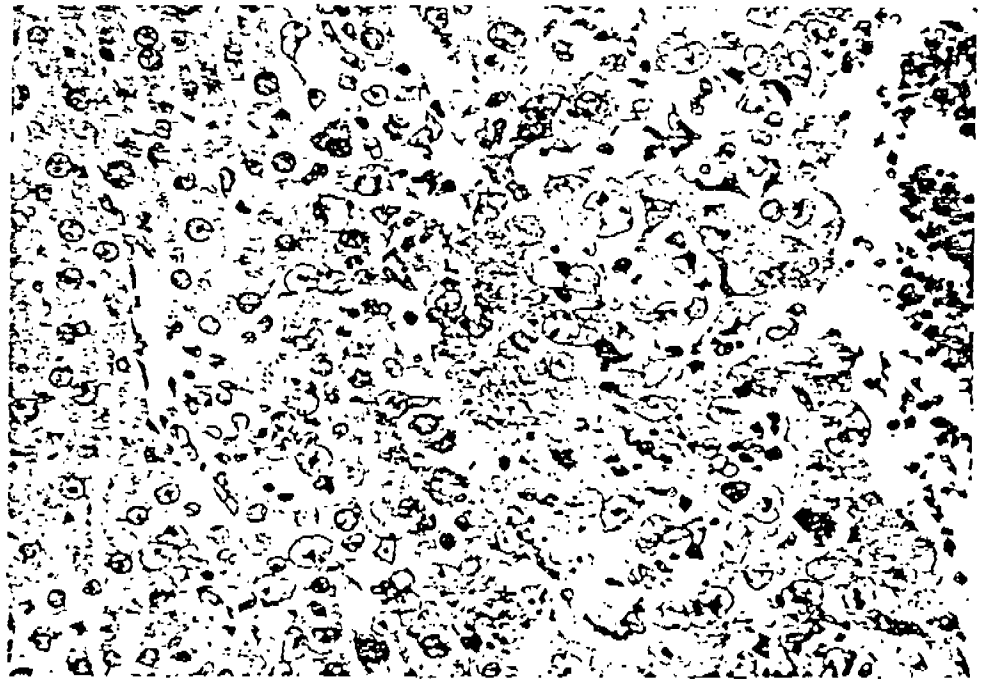

Fig. 4. Higher magnification of the hepatic angiosarcoma depicted in Fig. 3, showing large irregularly-shaped cells with big pale nuclei often containing multiple conspicuous nucleoli. Haematoxylin and eosin ×400.

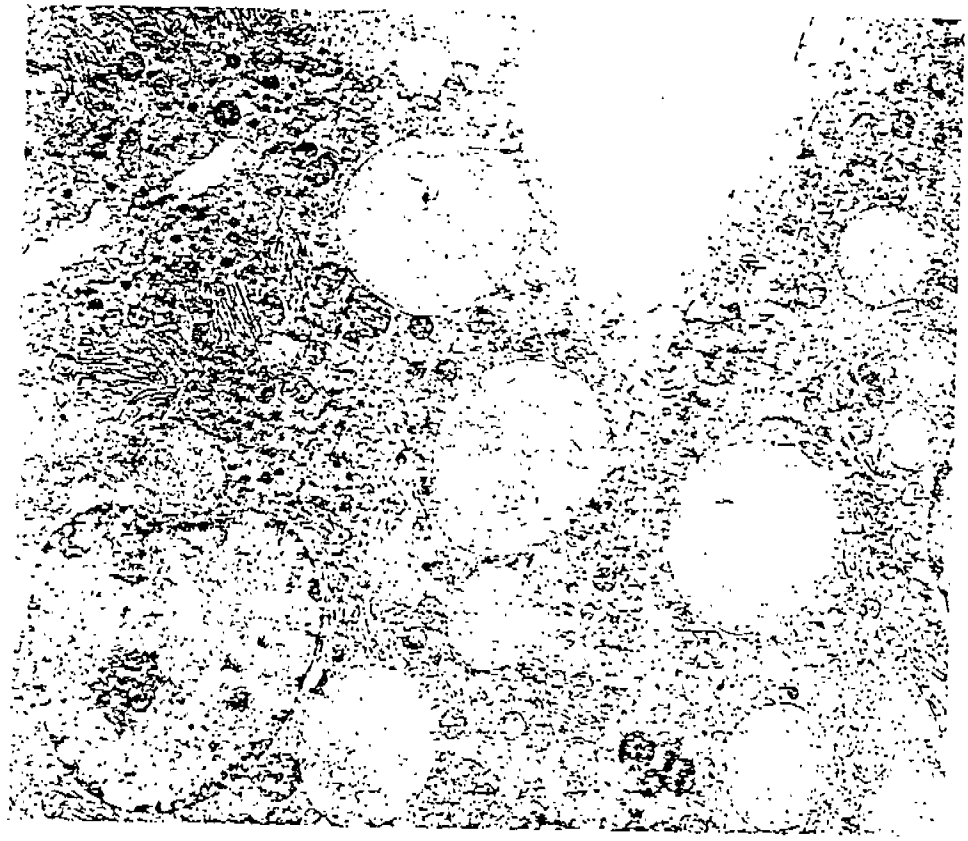

Fig. 5. Hepatocytes containing normal and highly swollen mitochondria with a pale matrix and short cristae from a male rat exposed orally to 300 mg VCM kg body weight on 5 days wk for 26 wk. Uranyl acetate, lead citrate ×6400.

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Table 5. *Site, type and incidence of tumours in organs other than the liver† in rats exposed orally to VCM for over 2·5 yr*

Site and type of tumour	Treatment group (mg VCM/kg/day)...	Incidence of tumours									
		Males					Females				
		0	1·7	5·0	14·1	300‡	0	1·7	5·0	14·1	300‡
	Effective no. of rats...	55	58	56	59	55	57	58	59	57	54
	No. of rats with *primary* tumours...	38	50	49	52	44	54	56	55	57	47
Lungs											
Angiosarcoma		0	0	4*	19***	19	0	0	1	5*	23
Adenoma		0	0	0	0	1	0	0	0	0	0
Zymbal glands											
Squamous-cell carcinoma		0	0	2	0	1	0	0	0	0	1
Adenoma		0	0	0	0	0	0	0	0	0	1
Abdomen											
Mesothelioma		3	1	7	8	1	1	6*	3	3	0
Angiosarcoma		0	0	0	0	1	0	0	0	2	1
Fibrosarcoma		0	0	0	3	0	1	2	0	0	0
Osteosarcoma		0	0	0	0	1	0	0	0	1	0
Sarcoma		0	0	3	1	0	0	0	1	0	0
Reticulum-cell sarcoma		0	1	0	0	1	0	0	0	0	0
'Schwann-cell tumour'		0	0	0	0	0	0	1	0	0	0
Unclassified		0	0	0	0	0	2	0	0	0	0
Spleen											
Haemangioendotheliosarcoma		0	1	0	0	0	0	0	0	0	0
Lymphosarcoma		0	0	0	0	0	0	0	0	0	1
Nose											
Squamous-cell carcinoma		0	0	0	1	0	0	0	0	0	0
Brain											
Granular-cell myoblastoma		1	1	0	0	0	0	1	0	0	0
Oligodendroglioma		1	0	0	0	0	0	0	0	0	0
Plexus papilloma		0	0	0	0	0	0	1	0	0	0
Glial-cell tumour		0	0	2	0	1	0	0	0	0	0
Ependymoma		0	0	0	1	0	0	0	0	0	0
Mesodermal tumour		0	0	1	0	0	0	0	0	0	0
Pancreas											
Adenocarcinoma		0	0	0	0	0	1	2	1	0	0
Thorax											
Mesothelioma		1	0	0	0	0	0	0	0	0	0
Thyroid											
Parafollicular-cell adenoma		4	12*	10	3	3	7	10	3	2	0
Parafollicular-cell carcinoma		1	0	1	0	0	0	0	0	0	0
Follicular-cell adenoma		0	0	0	1	0	1	0	0	1	0

Table 5—*continued*

Site and type of tumour	Treatment group (mg VCM/kg/day) ..	Incidence of tumours									
		Males					Females				
		0	1·7	5·0	14·1	300‡	0	1·7	5·0	14·1	300‡
Adrenals											
Cortical adenoma		18	25	17	10	9	26	30	20	17	14
Phaeochromocytoma		11	21	8	4	6	2	1	2	0	2
Pituitary											
Adenoma		12	25**	6	2**	0	14	16	10	5*	3
Carcinoma		1	0	1	0	0	3	0	2	0	0
Blood											
Leukaemia		1	0	1	1	3	1	2	1	0	1
Heart											
'Endocardial disease'§		2	0	2	2	1	1	0	0	0	1
Haemangioendotheliosarcoma		1	0	0	0	0	0	0	0	0	0
Kidneys											
Nephroblastoma		1	0	0	1	0	0	0	0	0	0
Clear-cell tumour		0	0	0	0	0	0	0	0	1	0
Lipomatous tumour		0	0	1	0	0	0	0	0	0	0
Unclassified epithelial tumour		0	0	0	0	0	1	0	0	0	0
Thymus											
Fibrosarcoma		0	1	0	0	0	0	0	0	0	0
Reticulum-cell sarcoma		0	0	0	0	1	0	1	1	0	0
Mesenteric lymph nodes											
Reticulum-cell sarcoma		0	0	0	0	1	0	0	0	0	0
Skin											
Squamous-cell carcinoma		2	3	3	1	0	0	0	0	1	0
Subcutis											
Fibroma		2	1	1	1	1	3	3	1	0	0
Fibrosarcoma		0	1	1	0	0	1	1	0	0	0
Mesenchymal tumour		0	0	0	0	0	1	0	0	0	0
Skeletal muscle											
Rhabdomyosarcoma		0	0	0	1	1	1	0	0	0	0
Skull											
Osteoma		1	0	0	0	0	0	0	0	0	0
Mesenchymal tumour		0	0	0	0	0	0	1	0	0	0
Ear region											
Adenocarcinoma of unknown origin		0	0	1	0	0	0	0	0	0	0
Urinary bladder											
Unclassified epithelial tumour		0	0	1	0	0	0	0	0	0	0
Preputial glands											
Squamous-cell carcinoma		0	0	1	0	0	0	0	0	0	0

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Urinary bladder										
[illegible]	0	0	1	0	0	0	0	0	0	0
	0	0	1	0	0	0	0	0	0	0
Mammary glands										
Adenoma	0	0	0	0	0	0	0	0	2	0
Fibroadenoma	0	0	0	0	0	21	25	12**	4***	7
Adenocarcinoma	0	1	0	2	0	3	2	4	7	7
Anaplastic carcinoma	0	0	0	0	0	0	0	1	0	0
Testes										
Interstitial-cell tumour	3	0	0	1	1					
Uterus										
Adenocarcinoma						6	3	1	1	0
Malignant fibroadenomatous tumour						0	1	0	0	0
Leiomyoma						0	0	1	0	0
Cervix										
Mesenchymal type of tumour						2	0	1	0	0
Adenocarcinoma						0	1	0	0	0
Ovaries										
Theca-cell tumour						0	0	1	0	0

†A small number of primary liver tumours unrelated to treatment were found in several groups. These tumours were one Kupffer-cell sarcoma, three reticulum-cell sarcomas, two fibrosarcomas, one haemangioendothelioma and one mesenchymal tumour.

‡The figures for this group were not evaluated statistically, because no corresponding control group was included in the study.

§In several cases the neoplastic character of the lesion was doubtful.

Values marked with asterisks differ significantly from those of the controls according to the chi-square test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

300-mg/kg groups than in controls or low-dose animals (Table 5). The low incidence of this common type of 'spontaneous' tumour, which is associated with old age, at the three highest dose levels is undoubtedly connected with the much shorter survival time of the animals in these groups compared with that of the controls or low-dose animals. Despite the relatively short survival time of females in the 14·1- and 300-mg/kg groups, the incidence of adenocarcinomas of the mammary glands in each of these groups was twice as high as in the control group. This may indicate that VCM enhances the development of mammary-gland carcinomas.

The incidence of several other common types of tumour known to be associated with old age was found to decrease with increasing dose levels, as was to be expected from the dose-related reduction in survival time. Examples of these were cortical adenomas and phaeochromocytomas of the adrenals, pituitary-gland adenomas, parafollicular-cell adenomas of the thyroid (the incidence of this tumour was exceptionally low in males of the control group), and adenocarcinomas of the uterus.

DISCUSSION

The limited daily feeding period of 4 hr in the daytime appeared to be associated with lower body weights but also with a higher survival rate. Apparently, reduced body-weight gain caused by a restricted feeding period is a favourable rather than an unfavourable effect. The much higher survival rate also points to the rats being healthy and having an adequate nutritional status unaffected by the relatively short feeding period in the day-time.

Only slight and often inconsistent differences were found between controls and test animals in several of the haematological and biochemical parameters studied. These slight deviations were regarded as toxicologically insignificant, with two possible exceptions, the shortening of the blood-clotting time and the increase in α-foetoprotein levels in the blood serum of VCM-exposed rats. Failure of the blood to clot was noted in guinea-pigs that died during exposure to an atmosphere containing 40% VCM (Mastromatteo, Fischer, Christie & Danziger, 1960) and in two workers in a PVC factory who died from acute VCM poisoning (Danziger, 1960). However, rats exposed to 5 or 2% VCM for 19 or 92 days, respectively, had normal blood-clotting times (Lester, Greenberg & Adams, 1963). In contrast, both in the present study and in a previous long-term inhalation study with VCM in rats (Feron, Kruysse & Til, 1979a), prothrombin times were shorter in VCM-exposed rats than in controls. Because of the conflicting observations, it is difficult to assess the toxicological importance of the slight hypercoagulability of the blood seen in the rats exposed to VCM in our studies. Müller, Buchter, Gross & Bolt (1976) recently reported a disturbed thrombocytic function in nine of 17 patients suffering from 'vinyl chloride disease'. Thrombocytopenia, which has been reported to be one of the signs of VCM intoxication in man (Jühe, Lange, Stein & Veltman, 1973; Lange, Jühe, Stein & Veltman, 1974; Müller *et al.* 1976), was observed neither in the present oral experiment nor in the previous 1-yr inhalation study (Feron *et al.* 1979a). In future studies, it would be desirable to pay special attention to the possible effects of VCM on thrombocytes.

Slight, though statistically significant, increases in the α-foetoprotein content of the blood serum were found in males and females of the 14·1-mg/kg group at wk 52. At wk 26 α-foetoprotein levels were normal in animals of this group. Very similar results have been obtained in rats exposed to 5000 ppm VCM in air for periods varying from 4 to 52 wk (Feron *et al.* 1979a). These findings may indicate the presence of foetoglobulin-producing neoplastic or preneoplastic cells in the liver of rats exposed to VCM for a prolonged period. However, since the increase in α-foetoprotein level was only slight, aspecific liver damage, such as necrosis or degeneration of hepatocytes, may have been responsible for this slight effect.

Although hepatocellular tumours have been reported in rats treated with VCM (Maltoni, 1977; Williamson, 1976) and some evidence exists as to the occurrence of hepatocellular carcinomas in persons exposed to VCM (Berk, Martin, Young, Creech, Selikoff, Falk, Watanabe, Popper & Thomas, 1976; Popper, Thomas, Telles, Falk & Selikoff, 1978), the high incidence of neoplastic liver-cell nodules and hepatocellular carcinomas in test animals (especially females) receiving VCM orally in PVC powder was an unexpected finding in the present study. Even at the lowest level (1·7 mg VCM/kg body weight·day), VCM-related liver-cell tumours and an increased incidence of foci of cellular alteration were noticeable in both males and females. It was remarkable that only a few hepatocellular neoplasms occurred in animals receiving VCM in oil by gavage at the very high dose of 300-mg/kg body weight, whereas nearly 50% of these rats had a hepatic angiosarcoma. The nature of the tumour response of the liver in the various test groups suggests a shift from an almost exclusive development of angiosarcomas at the very high dose level, *via* both angiosarcoma and hepatocellular tumours at the intermediate dose levels, to the exclusive occurrence of liver-cell tumours at the lowest level. On the other hand, not only the dose but also the way of administering VCM may have been of significance for the difference in tumour response between the 300-mg/kg group and the lower dosage groups, because gastric intubation of VCM in oil (daily on 5 days/wk) implies that the body (the liver) has to deal with a large amount of VCM in a short time, whereas in the case of oral intake of VCM-containing PVC powder the body (the liver) is continuously exposed (most probably for 24 hr/day on 7 days/wk) to 'fresh' VCM released gradually and without interruption from the PVC powder during its transport through the gastro-intestinal tract. One may also speculate that the unexpectedly high incidence of liver-cell tumours in the groups receiving VCM in PVC powder could be due to an unusual sensitivity of the liver cells to VCM, developing as a consequence of an altered metabolic state caused by the reduction of the daily period of food intake to 4 hr. This restricted period of food intake may have caused a daily, transitory depression of hepatic glutathione, which has been demonstrated to be of significance for the detoxification of VCM or its reactive metabolites

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(Watanabe, Hefner & Gehring, 1976; Watanabe, McGowan & Gehring, 1976; Watanabe, McGowan, Madrid & Gehring, 1976).

The lesions of the hepatic parenchyma that could be attributed to VCM included not only 'foci of cellular alterations', neoplastic nodules and carcinomas, but also necrosis, centrilobular degeneration and mitochondrial damage. In addition, the slight increases in the α-foetoprotein content of the blood and in relative liver weight may also have been indications of VCM injury to the hepatic parenchymal cells. Since it is known that tissue injury followed by repair (hyperplasia) may stimulate tumour formation, the possibility exists that the 'promoting' properties of this chronic process of continuous damage and repair play a major role in the development of hepatocellular tumours following VCM exposure.

The association between VCM and angiosarcoma in man was recognized some years ago (Creech & Johnson, 1974; Lloyd, 1975) and several investigators have produced angiosarcomas in experimental animals by exposure to VCM either by inhalation or oral administration (Feron *et al.* 1979b; Holmberg, Kronevi & Winell, 1976; Keplinger, Goode, Cordon & Calandra, 1975; Lee *et al.* 1978; Maltoni, 1975 & 1977). The results of the present experiment show that oral exposure of rats to VCM at levels of 5·0 mg/kg body weight/day or more caused hepatic angiosarcomas, pulmonary angiosarcomas (probably both primary tumours and metastases) and, at the higher levels, also a few primary extrahepatic abdominal angiosarcomas. No angiosarcomas were found at the lowest level of 1·7 mg VCM/kg body weight/day.

Ultrastructural studies of the liver showed a scattering of foci of parenchymal cells affected by VCM. Swelling of mitochondria was the earliest change observed and mitochondrial alterations were also characteristic of the VCM-induced damage seen at later stages. Swelling of mitochondria has been found after exposure to other carcinogens (Bannasch, 1975) and also under various pathological conditions, such as ethanol damage (Dobbins, Rollins, Brooks & Fallon, 1972) and riboflavin deficiency (Tandler, Erlandson & Wynder, 1968). In addition to mitochondrial swelling, prolonged treatment with VCM for 26 or 52 wk appeared to cause a decrease in and loss of ribosomes from the RER, and most importantly an increase in SER. These findings suggest an enhancing effect of VCM on the so-called mixed-function oxidase system (m.f.o. system) which has been shown to be involved in the detoxification of VCM (Bolt, Kappus, Bruchter & Bolt, 1976; Reynolds, Moslen, Szabo, Jaeger & Murphy, 1975). However, the determinations of aminopyrine-demethylase and aniline-hydroxylase activities in the liver did not produce evidence of m.f.o. induction by VCM.

Full development of VCM-induced angiosarcoma has been found to be preceded by nodular hypertrophy and hyperplasia of liver cells, which may proceed to hepatocellular carcinoma (Feron *et al.* 1979b; Popper, Selikoff, Maltoni, Squire & Thomas, 1977). The liver changes found in the three lowest dosage groups are well in line with this observation, but in this connection it is difficult to understand the high incidence of hepatic angiosarcomas in the 300-mg/kg group, because focal hypertrophy and hyperplasia of the parenchyma occurred in only a very limited number of rats of this group.

Several investigators (Lee *et al.* 1978; Maltoni, 1975) have found extrahepatic angiosarcomas in rats exposed to VCM. In the present oral study, extrahepatic angiosarcomas were also seen, although it should be stressed that most of the pulmonary angiosarcomas were considered to be metastases from hepatic angiosarcomas.

Only a few test animals bore a tumour of the Zymbal glands, but since spontaneous Zymbal-gland tumours are rare and tumours of this organ are known to be induced in rats by VCM, it seems justifiable to ascribe to the VCM treatment the few Zymbal-gland neoplasms found.

VCM-induced carcinomas of the mammary glands have been observed in mice (Lee *et al.* 1978; Maltoni, 1975) and presumably also in rats (Maltoni, 1975). The incidence of mammary-gland carcinomas in several of the test groups was only slightly higher than that in the controls and was not considered to be conclusive evidence that VCM can induce this type of neoplasm in rats. At most it may be regarded as an indication that VCM may enhance carcinoma formation in this organ.

To our knowledge, VCM-induced mesotheliomas have not been reported. In the present study, the number of rats with abdominal mesothelioma was higher in each of the test groups receiving PVC-powder containing VCM than in controls fed PVC powder free of VCM. This may indicate that the ingestion of VCM has a potentiating effect on the development of mesothelioma in rats.

The high incidence of liver-cell tumours in females of the low-dose group (28/58) clearly demonstrates the absence of both a 'no-observed-adverse-effect level' and a 'minimum-effect level' in our experimental system. These levels must be known before any attempt can be made to extrapolate the animal data to man and to assess the carcinogenic risk of VCM. Therefore, a similar lifespan oral carcinogenicity study with VCM in rats has been initiated, using three different dose levels (0·017, 0·17 and 1·7 mg VCM/kg body weight/day) and two control groups; this study had been running for 14 months in October 1980.

Conclusions

This study shows that the incorporation of VCM-containing PVC powder into the diets of rats is an effective method for studying the long-term effects of oral administration of VCM. VCM is a carcinogen when administered to rats by the oral route and the tumour response of the rat liver to the oral intake of VCM seems to shift from an almost exclusive development of angiosarcomas at very high levels to the exclusive induction of hepatocellular tumours at low levels of exposure. The ingestion of VCM in PVC powder may enhance in rats the development of abdominal mesotheliomas and of adenocarcinomas of the mammary glands. The 'no-observed-adverse-effect level' was lower than 1·7 mg VCM/kg body weight/day under the rigorous conditions of continuous oral VCM exposure resulting from the gradual release of VCM from PVC powder present in the gastrointestinal tract.

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REFERENCES

Ministry of Agriculture, Fisheries and Food (1978). The Surveillance of Food Contamination in the United Kingdom: Survey of Vinyl Chloride Content of Polyvinyl chloride for Food Contact and of Foods. The Second Report of the Steering Group on Food Surveillance—The Working Party on Vinyl Chloride. Food Surveillance Paper No. 2, p. 6. HMSO, London.

Bannasch, P. (1975). Die Cytoplasmaorganellen der Hepatocyten während der Carcinogenese. 1. Mitochondrien. In *Handbuch der allgemeinen Pathologie. Sechster Band, siebenter Teil, Geschwülste-Tumors III*. Edited by E. Grundmann, p. 211. Springer Verlag, Berlin.

Basalaev, A. V., Vazin, A. N. & Kotchetkov, A. G. (1972). On the pathogenesis of changes developing due to a long-term exposure to the effect of vinyl chloride. *Gig. Truda prof. Zabol.* 16, 24.

Berk, P. D., Martin, J. F., Young, R. S., Creech, J., Selikoff, I. J., Falk, H., Watanabe, P., Popper, H. & Thomas, L. (1976). Vinyl chloride-associated liver disease. *Ann. intern. Med.* 84, 717.

Bessey, O. A., Lowry, O. H. & Brock, M. J. (1946). A method for the rapid determination of alkaline phosphatase with five cubic millimetres of serum. *J. biol. Chem.* 164, 321.

Boekestein-Tjahjadi, H. M. & Kroes, R. (1976). α_1-Fetoproteine (AFP): Radioimmunologische Bepaling en de Vroege Verschijning van APF na Aflatoxine B_1 Toediening. Berichten uit het RIV-1975, p. 136. Staatsuitgeverij, Den Haag.

Bolt, H. M., Kappus, A., Bruchter, A. & Bolt, W. (1976). Disposition of (1,2-^{14}C) vinyl chloride in the rat. *Archs Toxicol.* 35, 153.

Ceriotti, G. & Spandrio, L. (1965). Catalytic acceleration of the urea-diacetylmonoxime-phenazone reaction and its application to automated analysis. *Clinica chim. Acta* 11, 519.

Creech, J. L., Jr & Johnson, M. N. (1974). Angiosarcoma of the liver in the manufacture of polyvinyl chloride. *J. occup. Med.* 16, 150.

Daniels, A. G. & Proctor, D. E. (1975). VCM extraction from PVC bottles. *Modern Packaging* April, p. 45.

Danziger, H. (1960). Accidental poisoning by vinyl chloride. *Can. Med. Ass. J.* 82, 828.

De Leeuw-Israël, F. R., Arp-Neefjes, J. M. & Hollander, C. F. (1967). Quantitative determination of albumin in microliter amounts of rat serum. *Expl Geront.* 2, 255.

Dobbins, W. O., Rollins, E. L., Brooks, S. G. & Fallon, H. J. (1972) A quantitative morphological analysis of ethanol effect upon rat liver. *Gastroenterology* 62, 1020.

Feron, V. J. & Kroes, R. (1979). One-year time-sequence inhalation toxicity study of vinyl chloride in rats. II. Morphological changes in the respiratory tract, ceruminous glands, brain, kidneys, heart and spleen. *Toxicology* 13, 131.

Feron, V. J., Kruysse, A. & Til, H. P. (1979a). One-year time sequence inhalation toxicity study of vinyl chloride in rats. I. Growth, mortality, haematology, clinical chemistry and organ weights. *Toxicology* 13, 25.

Feron, V. J., Speek, A. J., Willems, M. I., van Battum, D. & de Groot, A. P. (1975). Observations on the oral administration and toxicity of vinyl chloride in rats. *Fd Cosmet. Toxicol.* 13, 633.

Feron, V. J., Spit, B. J., Immel, H. R. & Kroes, R. (1979b) One-year time-sequence inhalation toxicity study of vinyl chloride in rats. III. Morphological changes in the liver. *Toxicology* 13, 143.

Fuchs, G., Gawall, B. M., Albanus, L. & Slorach, S. (1975). Vinyl chloride monomer levels in edible fats. *Vår Föda* 27, 134.

Gilbert, D. & Golberg, L. (1965). Liver response tests. III. Liver enlargement and stimulation of microsomal processing enzyme activity. *Fd Cosmet. Toxicol.* 3, 417.

Gorter, E. & De Graaff, W. C. (1955). *Klinische Diagnostiek. I.* 7th Ed. p. 267. Edited by H. E. Stenfert. Kroese NV, Leiden.

Gram, T. E., Wilson, J. T. & Fouts, J. R. (1968). Some characteristics of hepatic microsomal systems which metabolize aminopyrine in the rat and rabbit. *J. Pharmac. exp. Ther.* 159, 172.

Haley, T. J. (1975). Vinyl chloride: how many unknown problems? *J. Toxicol. envir. Hlth* 1, 47.

Holmberg, B., Kronevi, T. & Winell, W. (1976). The pathology of vinyl chloride exposed mice. *Acta vet. scand.* 17, 328.

Hopkins, J. (1980). Vinyl chloride—Part 4: Carcinogenicity in man. *Fd Cosmet. Toxicol.* 18, 94.

Jühe, S., Lange, C. E., Stein, G. & Veltman, G. (1973). Über die sogenannte Vinylchloride-Krankheit. *Dt. med. Wschr.* 98, 2034.

Keplinger, M. L., Goode, J. W., Cordon, D. E. & Calandra, J. C. (1975). Interim results of exposure of rats, hamsters, and mice to vinyl chloride. *Ann. N.Y. Acad. Sci.* 246, 219.

Lange, C. E., Jühe, S., Stein, G. & Veltman, G. (1974). Die sogenannte Vinylchloride-Krankheit—eine berufsbedingte Systemsklerose? *Int. Arch. Arbeitsmed.* 32, 1.

Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L. & Woods, J. S. (1978). Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. envir. Hlth.* 4, 15.

Lester, D., Greenberg, L. A. & Adams, W. A. (1963). Effects of single and repeated exposures of humans and rats to vinyl chloride. *Am. ind. Hyg. Ass. J.* 24, 265.

Lloyd, J. W. (1975). Angiosarcoma of the liver in vinyl chloride/polyvinyl workers *J. occup. Med.* 17, 333.

Maltoni, C. (1975). The value of predictive experimental bioassays in occupational and environmental carcinogeneses. An example: vinyl chloride. *Ambio* 4, 18

Maltoni, C. & Lefemine, G. (1975). Carcinogenicity bioassays of vinyl chloride: current results. *Ann. N.Y. Acad. Sci.* 246, 195

Maltoni, C. (1977). Vinyl chloride carcinogenicity: an experimental model for carcinogenesis studies. In *Origins of Human Cancer. Book A*. Edited by H. H. Hiatt, J. D. Watson & J. A. Winsten, p. 119. Cold Spring Harbor Laboratory, NY.

Mastromatteo, E., Fischer, A. M., Christie, H. & Danziger, H. (1960). Acute inhalation toxicity of vinyl chloride to laboratory animals *Am. ind. Hyg. Ass. J.* 21, 394.

Monson, R. R., Peters, J. M. & Johnson, M. N. (1974). Proportional mortality among vinyl-chloride workers. *Lancet* II, 397.

Müller, K. T., Buchter, A., Gross, R. & Bolt, W. (1976). Ergebnisse einer Studie an 17 Fällen von Langzeitexposition gegenüber Vinylchlorid. *Medsche Welt, Berl.* 27, 21.

Paschen, K. & Fuchs, C. (1971). A new micro-method for Na, K, Ca and Mg determinations in a single serum dilution by atomic absorption spectrophotometry. *Clinica chim. Acta* 35, 401.

Popper, H., Selikoff, I. J., Maltoni, C., Squire, R. A. & Thomas, L. B. (1977). Comparison of neoplastic hepatic lesions in man and experimental animals. In *Origins of Human Cancer*. Edited by H. H. Hiatt, J. D. Watson & J. A. Winsten, p. 1359. Cold Spring Harbor Laboratory, NY.

Popper, H., Thomas, L. B., Telles, N. C., Falk, H. & Selik-

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off, I. J. (1978). Development of hepatic angiosarcoma in man induced by vinyl chloride, thorotrast and arsenic: comparison with cases of unknown etiology. *Am. J. Path.* 92, 349.

Potter, H. R. (1976). Vinyl Chloride—Part II. *Fd Cosmet. Toxicol.* 14, 498.

Randolph, W. F. (1973). Prior-sanctioned polyvinyl chloride resin. *Federal Register* 38, 12934.

Rawls, R. (1980). Studies update vinyl chloride hazards. *Chem. Engng News* 58 (14), 27.

Reitman, S. & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic-oxalacetic and glutamic-pyruvic transaminases. *Am. J. clin. Path.* 28, 56.

Reynolds, E. S., Moslen, M. T., Szabo, S., Jaeger, R. J. & Murphy, S. D. (1975). Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene: role of mixed function oxidase system. *Am. J. Path.* 81, 219.

Sharratt, M. & Frazer, A. C. (1963). The sensitivity of function tests in detecting renal damage in the rat. *Toxic. appl. Pharmac.* 5, 36.

Squire, R. A. & Levitt, M. H. (1975). Report of a workshop on classification of specific hepatocellular lesions in rats. *Cancer Res.* 35, 3214.

Suzuki, Y. (1978). Pulmonary tumors induced in mice by vinyl chloride monomer. *Envir. Res.* 16, 285.

Tandler, B., Erlandson, R. A. & Wynder, E. L. (1968). Riboflavin and mouse hepatic cell structure and function. I. Ultrastructural alterations in simple deficiency. *Am J. Path.* 52, 69.

Thomas, L. B. & Popper, H. (1975). Pathology of angiosarcoma of the liver among vinyl chloride–polyvinyl chloride workers. *Ann. N.Y. Acad. Sci.* 246, 268.

Torkelson, T. R., Oyen, F. & Rowe, V. K. (1961). The toxicity of vinyl chloride as determined by repeated exposure of laboratory animals. *Am. ind. Hyg. Ass. J.* 22, 354.

Vale, P. T., Kipling, M. D. & Walker, A. E. (1976). Miscellaneous symptoms occurring in workers engaged in the manufacture of PVC. *J. Soc. occup. Med.* 26, 95.

van Kampen, E. J. & Zijlstra, W. C. (1961). Standardization of hemoglobinometry. II. The hemoglobincyanide method. *Clinica chim. Acta* 6, 538.

Watanabe, P. G., Hefner, R. E., Jr & Gehring, P. J. (1976). Vinyl chloride-induced depression of hepatic non-protein sulfhydryl content and effects on bromosulphalein (BSP) clearance in rats. *Toxicology* 6, 1.

Watanabe, P. G., McGowan, G. R. & Gehring, P. J. (1976). Fate of [^{14}C]vinyl chloride after single oral administration in rats. *Toxic. appl. Pharmac.* 36, 339.

Watanabe, P. G., McGowan, G. R., Madrid, E. O. & Gehring, P. J. (1976). Fate of [^{14}C]vinyl chloride following inhalation exposure in rats. *Toxic appl. Pharmac.* 37, 49.

Waxweiler, R. J., Stringer, W., Wagoner, J. K., Jones, J., Falk, H. & Carter, C. (1976). Neoplastic risk among workers exposed to vinyl chloride. *Ann. N.Y. Acad. Sci.* 271, 40.

Wieme, R. J. (1965). *Agar Gel Electrophoresis*. p. 425. Elsevier Publishing Corp., Amsterdam.

Williams, D. T. & Miles, W. F. (1975). Gas–liquid chromatographic determination of vinyl chloride in alcoholic beverages, vegetable oils and vinegars. *J. Ass. off. analyt. Chem.* 58, 272.

Williamson, K. S. (1976). Review of animal studies. *Proc. R. Soc. Med.* 69, 281.

Winell, M., Holmberg, B. & Kronevi, T. (1976). Biological effects of vinyl chloride: an experimental study. *Envir. Hlth Perspect.* 17, 211.

Wroblewski, F. & LaDue, J. S. (1955). Lactic dehydrogenase activity in blood. *Proc. Soc. exp. Biol. Med.* 90, 210.