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S D Gangolli

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LIFETIME (149-WEEK) ORAL CARCINOGENICITY STUDY OF VINYL CHLORIDE IN RATS*

H. P. TIL, V. J. FERON and H. R. IMMEL

TNO Toxicology and Nutrition Institute, PO Box 360, 3700 AJ Zeist, The Netherlands

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Abstract—A lifetime (149-wk) oral carcinogenicity study of vinyl chloride monomer (VCM) was carried out. Four groups of Wistar rats were used, each consisting of 100 males and 100 females, except for the high-dose group, which comprised 50 males and 50 females. VCM was administered by incorporating polyvinyl chloride powder with a high content of VCM into the diet. The actual exposure levels of VCM were 0 (control), 0.014, 0.13 and 1.3 mg VCM/kg body weight/day. Detailed histopathological examination was restricted to the liver. In the final stage of the study, the mortality in the high-dose group was slightly higher than in controls. A variety of VCM-related liver lesions was found in the high-dose group. The lesions included increased incidences of liver-cell polymorphism, hepatic cysts, foci of cellular alteration, neoplastic nodules, hepatocellular carcinomas and angiosarcomas. Compared with controls, there were increased incidences of hepatic foci of cellular alteration in females of the mid-dose group and of basophilic foci of hepatocellular alteration in females of both the low- and mid-dose groups. There was no evidence that feeding of VCM affected the incidence of tumours in organs other than the liver. Thus, the present study showed that the feeding of VCM at a level of 1.3 mg/kg body weight/day can induce neoplastic and non-neoplastic changes in the livers of male as well as female rats. The feeding of 0.014 or 0.13 mg VCM/kg body weight/day may result in an increased incidence of (basophilic) foci of cellular alteration in the liver of female rats. It was concluded that 0.13 mg VCM/kg body weight/day is the no-observed-adverse-effect level with respect to the induction of tumours in rats.

INTRODUCTION

When, in the early 1970s, angiosarcomas were found in the livers of workers at vinyl chloride monomer (VCM) and polyvinyl chloride (PVC) production plants, drastic measures were taken to reduce VCM exposure in the workplace. By changing production processes and plant installations and by implementing new production processes, worldwide VCM exposure in the workplace could be reduced to 1 ppm.

Great efforts were also made for consumers' safety, because it was feared that the residual VCM in PVC packaging materials could be indirectly ingested by humans by way of the PVC-packaged foodstuffs. Therefore, in addition to inhalation studies, mainly aimed at the protection of plant workers, feeding studies were performed. The aims of the latter studies were to examine whether the administration route would influence the effect of VCM, and to establish the oral no-adverse-effect level of VCM. A 28-day feeding study in rats with doses of 0, 90 and 300 mg VCM/kg body weight/day served as the preliminary experiment for a 90-day study in rats receiving oral doses of 0, 30, 100 and 300 mg VCM/kg body weight/day by gavage. A no-effect level of 30 mg/kg

body weight/day was determined (Feron *et al.*, 1975). These short-term studies were soon followed by a chronic oral toxicity/carcinogenicity study†. The difficulty in incorporating such a volatile gas as VCM into the diet resulted in a higher actual VCM intake than intended; thus, in this study, 1.7 instead of 1 mg was used for the low dose, 5 instead of 3 mg for the mid dose, and 14.1 instead of 9 mg/kg body weight/day was used for the high dose. An extra group received 300 mg/kg body weight/day by gavage, 5 days/wk for 83 wk (Feron *et al.*, 1981). From this study, it appeared that hepatic angiosarcomas occurred at doses of 5 mg/kg body weight/day and above, while hepatocellular tumours were found at all doses including the lowest level of 1.7 mg/kg body weight/day. Tumours attributable to VCM exposure and found at other sites included pulmonary angiosarcomas, extrahepatic abdominal angiosarcomas and Zymbal-gland tumours; these neoplasms occurred at VCM levels of 5 mg/kg body weight and above. Moreover, some evidence was found that VCM enhanced the development of abdominal mesotheliomas and of mammary-gland adenocarcinomas. Thus, this study showed that VCM is a carcinogen in rats when administered orally, and that the no-observed-adverse-effect level of VCM in rats with respect to the induction of tumours was lower than 1.7 mg/kg body weight/day. It was noted that the tumour response in this study might have been influenced by the special route of administration of VCM (continuous slow release of VCM from the PVC granules in the gastro-intestinal tract) and by the limited period of time (4 hr/day) that the rats had access to their diets.

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Abbreviations: PVC = polyvinyl chloride; VCM = vinyl chloride monomer.

For accurate extrapolation of experimental data to humans, the information obtained from such a test system should ideally include both a minimum-tumour level and a no-tumour level. Therefore, in the early 1980s, a similar lifetime oral carcinogenicity study of VCM in rats was carried out, including lower dose levels of 0.014, 0.13 and 1.3 mg VCM/kg body weight/day. Based on the pathological findings of the previous long-term study, histopathological examinations in this experiment were focused on the detection of liver lesions, Zymbal-gland tumours, mammary-gland carcinomas and abdominal mesotheliomas. This study is described in the present paper.

MATERIALS AND METHODS

Test materials. VCM was obtained from AKZO Zout Chemie (Rotterdam, The Netherlands) and PVC powder was supplied by Shell Nederland Chemie (Pernis, The Netherlands). Details of these materials were given in an earlier paper (Feron *et al.*, 1981). A portion of this PVC powder was separated from residual VCM and was used for incorporation into the diet of the control animals. 50 kg PVC powder was mixed with liquid VCM up to a level of approximately 4600 ppm in a closed steel barrel on a roller-bank. This PVC powder was repacked and stored in tightly closed steel drums (each containing about 10 kg PVC) in a freezer at -20°C until a few min before mixing into the diet.

Diets. Preparation and administration of the diets were carried out as described previously (Feron *et al.*, 1981). In the present study, however, the diets contained only 1% PVC powder. For the treated groups, the PVC powder enriched with VCM was mixed into the stock diet to provide concentrations of 0.46, 4.6 and 46 ppm. PVC powder without VCM (lower than 0.2 ppm) was added to the diets such that the total amount of PVC powder in each diet was 1%. The diets were prepared daily, immediately prior to being offered to the rats. As a result of the evaporation of VCM, diets were available to the rats each day for a period of 4 consecutive hr only (generally between 10 am and 2 pm). The VCM content of freshly prepared diets was determined once every fortnight; a total of 72 determinations for each dosage level was carried out during the study. From these determinations the average VCM contents of the diets were calculated to be 0.49, 4.49 and 44.1 ppm for the low-, mid- and high-dose groups, respectively. Since both the loss of VCM from the diets during the 4-hr period

and the VCM content of the faeces were determined, as described previously by Feron *et al.* (1981), the actual exposure levels of VCM could be calculated. These levels were found to be 0.014, 0.13 and 1.3 mg VCM/kg body weight/day for the low-, mid- and high-dose groups, respectively (Table 1).

Animals and housing. Newly weaned albino rats (Cpb: WU; Wistar random), obtained from the SPF colony of the Central Institute for the Breeding of Laboratory Animals (TNO, Zeist, The Netherlands), were used. Healthy rats were randomly allocated to four main groups. Three of the groups—the control, low- and mid-dose groups—each consisted of 100 males and 100 females. The high-dose group consisted of 50 males and 50 females. After allocation, the rats were maintained on a basal diet for 4–6 hr each day, and given tap-water continuously for 7 days prior to the start of the study. The rats were housed under conventional conditions, in groups of five in suspended stainless-steel cages with wire-screen bottoms. Male rats of each group were housed in one room, and females were housed in a separate room. The rooms were ventilated with approximately 10 air changes/hr. The room temperature was kept at $23 \pm 1^{\circ}\text{C}$ and the humidity was 40–80%, with a 12-hr light/dark cycle. Rats in bad condition were housed individually in separate cages until they died or were killed *in extremis*.

Experimental protocol. The rats were individually weighed, initially at wk 2 and 4, and once every 4 wk thereafter. Food consumption of 20 rats/sex/group was measured during wk 1–4 and 11–12, and then every 12 wk for periods of 2 wk (e.g. wk 24–26, 36–38, etc.). All males still alive at wk 149 and all females surviving until wk 150 were killed *in extremis*. Samples of a large number of organs and tissues of each rat were fixed in 10% neutral buffered formalin. The organs to be examined microscopically were processed through paraffin wax, sectioned at $5 \mu\text{m}$ and stained with haematoxylin and eosin. Microscopic examination was restricted to the liver and to all grossly visible tumours or suspected tumours in the abdominal cavity, Zymbal glands and mammary glands. For each rat, three pieces of liver, taken from three different lobes (the same lobes and the same sites of every rat), were examined microscopically. In addition, sections were prepared from liver tissue showing gross changes.

Glutathione levels of the liver. Additional groups of 10 male and 10 female rats received 0, 0.014, 0.13 and 1.3 mg VCM/kg body weight/day. In wk 40 and 80, five rats/sex/group were killed by decapitation. The

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

Designed VCM treatment			Oral intake of VCM		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† (mg/kg body weight/day)	Actual (mg/kg body weight/day)	
0	0	0	0	0	0
0.46	0.017	0.49	0.022	0.018	0.014
4.6	0.17	4.49	0.21	0.17	0.13
46	1.7	44.1	2.1	1.7	1.3

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

†Assuming no loss of VCM by evaporation from the diets.

‡Oral intake of VCM diminished by the faecal VCM. 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.

Table 2. Cumulative mortality of rats orally exposed to VCM for up to 149 wk

Treatment group (mg VCM/kg body weight/day)	Initial no of rats	No. of dead rats by end of wk:									
		16	36	52	72	88	104	116	128	140	149
Males											
0	100	1	1	2	4	6	13	21	38	59	80
0.014	100	1	1	1	4	8	15	25	43	63	80
0.13	100	0	0	1	4	7	13	23	38	59	82
1.3	50	0	0	0	1	2	7	18	24	35	42
Females											
0	100	0	0	1	2	6	15	26	35	58	76
0.014	100	0	0	0	2	4	11	25	41	66	77
0.13	100	0	0	0	3	4	14	22	35	61	74
1.3	50	0	0	0	2	6	12	16	24	36	45*

The value marked with an asterisk differs significantly (Fisher's exact probability test, one-tailed) from the corresponding control value (* $P < 0.05$).

livers were quickly removed for the determination of the glutathione content, according to an automated modification of the method of Sedlak and Lindsay (1968).

RESULTS

Clinical signs and survival

No clinical signs attributable to VCM treatment were apparent in the low- and mid-dose groups. Nodules in the liver were palpated in a high number of high-dose females. Mortality was very low up to wk 72 (Table 2). Thereafter, mortality gradually increased, and at the end of the study the mortality rate was slightly higher in the high-dose group than in the other groups.

Body weights and food consumption

Body weights (Fig. 1) and food intake were very similar for all groups.

Glutathione levels of the liver

There were no significant differences in liver glutathione levels among the various groups, either after wk 40 or 80.

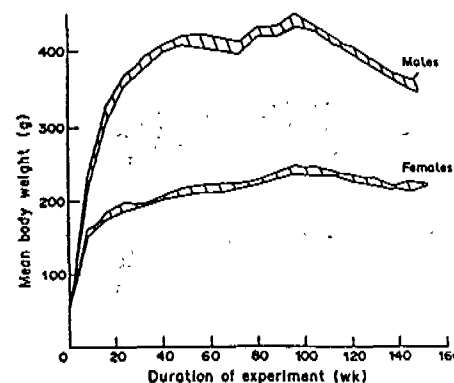


Fig. 1. Average body weights of rats orally exposed to VCM. The body weight curves of the rats receiving 0, 0.014, 0.13 or 1.3 mg VCM/kg body weight/day all lie within the shaded areas.

Gross pathology

The type and incidence of gross findings in the liver are given in Table 3. The incidence of liver nodules suspected of being tumours was higher in males and females of the high-dose groups than in controls or lower-dose rats. Many of these tumorous masses were small (up to 1 cm dia.), solid and pale, or had the same colour as the adjacent liver tissue; some were large (2–4 cm dia.), soft or firm, and occasionally slightly haemorrhagic. The appearance of cysts in the livers of old rats, particularly in females, is a common finding in the strain of rat used. However, the incidence of such cysts was much higher in females of the high-dose group than in females of the other groups, including the control group. The cysts varied widely in size, were often multiple and generally contained a turbid, watery liquid. There was no evidence that any of the other gross lesions observed were related to the administration of VCM.

Microscopy of the liver

The type and incidence of treatment-related histopathological changes found in the liver are given in Table 4. Increased incidences of foci of cellular alteration, neoplastic nodules, hepatocellular carcinomas, liver-cell polymorphism and cysts occurred in the high-dose group. Moreover, in this group two females and one male had developed a hepatic angiosarcoma, whereas such tumours were not seen in any of the other groups. Since the morphology of the hepatocellular lesions was essentially the same as that of the liver lesions described in full detail in our previous publication on the chronic effects of oral administration of VCM in rats (Feron *et al.*, 1981), no further description of these alterations is presented in this paper. The morphology of VCM-induced hepatic angiosarcomas has also been reported by Spit *et al.* (1981). Table 4 also shows that in females but not in males of both the low- and mid-dose groups, the incidence of basophilic foci of cellular alteration was significantly higher than in controls. In addition, in the mid-dose group the number of females showing foci of cellular alteration was significantly higher than in the control group.

Other hepatic lesions observed included prominent sinusoidal cells, bile-duct proliferation, cholangiofibrosis, focal vacuolization of hepatocytes, accumulation of RES cells, focal necrosis, individual cell necrosis and centrilobular liver-cell degeneration.

Table 3. Type and incidence of macroscopic changes in the liver of rats orally exposed to VCM

Type of change	Treatment group (mg VCM/kg body weight/day) ...	Incidence of change							
		Males				Females			
		0	0.014	0.13	1.3	0	0.014	0.13	1.3
Initial no. of rats		100	100	100	50	100	100	100	50
Effective no. of rats		99	100	99	49	98	100	96	49
<i>Liver</i>									
Tumour or suspected tumour		1	1	2	7***	2	1	3	8**
Prominent lobular pattern		1	4	2	0	0	1	3	1
Swollen/enlarged		2	4	5	1	3	2	0	0
Discoloured (a) pale		3	2	0	1	1	3	1	2
(b) dark		3	3	0	0	0	0	0	1
Cyst(s)		2	1	5	4	17	14	22	33***
Small		0	1	1	0	0	0	2	0
Small surface lesions (rosette, constriction)		1	0	1	0	3	0	0	0
Spotted		5	6	8	2	2	1	6	2
Granular surface		2	0	0	2	0	1	2	0

Values marked with asterisks differ significantly (chi-square test) from the corresponding control value (** $P < 0.01$; *** $P < 0.001$).

There was no evidence that any of these lesions was related to the feeding of VCM.

Microscopy of organs other than the liver

There was no evidence that the incidence of mammary-gland or intra-abdominal tumours was related to the administration of VCM. No Zymbal-gland tumours were found.

DISCUSSION

In this supplementary study no effects attributable to VCM were observed other than liver changes, and a slightly higher mortality in the high-dose group

compared with the control group during the last 6-9 months of the experimental period. The increased mortality in the high-dose group might be due to the increased incidence of neoplastic and non-neoplastic liver changes in this group. In the previous long-term oral rat study with VCM (Feron *et al.*, 1981) a similar adverse effect on survival was observed in females receiving 1.7 mg VCM/kg body weight/day.

Feron *et al.* (1981) reported that ingestion of VCM at a level of 1.7 mg/kg body weight/day resulted in a number of hepatic changes. These changes included an increased incidence of foci of cellular alteration and of neoplastic nodules, a few hepatocellular carcinomas, an increased incidence and degree of liver-cell

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

Type of change†	Treatment group (mg VCM/kg body weight/day) ...	Incidence of change							
		Males				Females			
		0	0.014	0.13	1.3	0	0.014	0.13	1.3
No. of rats examined ...		99	99	99	49	98	100	96	49
<i>Foci of cellular alteration</i>									
Clear-cell foci									
One or a few		12	8	8	16**	4	5	3	13***
Several		0	0	0	3*	0	0	0	6***
Basophilic foci									
One or a few		4	2	3	8*	9	20*	26***	19***
Several		0	0	0	0	0	1	0	2
Mixed cell foci									
One or a few		0	1	2	2	6	6	4	8*
Eosinophilic foci									
One or a few		1	1	2	1	0	0	1	10***
All types of foci (total)		17	12	15	30	19	32	34	58
No. of foci-bearing rats ...		16	12	15	23***	19	27	31*	32***
<i>Neoplastic nodules</i>									
One		0	0	0	1	0	1	1	9***
Few		0	0	0	2	0	0	0	1
Hepatocellular carcinoma		0	0	0	3*	1	0	1	3
Angiosarcoma		0	0	0	1	0	0	0	2
<i>Liver-cell polymorphism</i>									
Slight		27	23	26	19	46	41	49	23
Moderate		4	4	7	10**	14	13	8	15*
Severe		1	1	1	3	2	3	4	9***
<i>Cysts</i>									
One		1	0	1	0	3	2	4	1
Few		4	4	3	4	11	11	12	7
Many		0	0	0	0	3	4	9	24***

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

Values marked with asterisks differ significantly (Fisher's exact probability test, one-tailed) from the corresponding control value (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

polymorphism, and an increase in the number hepatic cysts. In the present study the same type of treatment-related liver changes were found at 1.3 mg VCM/kg body weight/day. In addition, one male and two females of the high-dose group developed a hepatic angiosarcoma; in the previous experiment hepatic angiosarcomas were observed at VCM levels of 5 mg/kg body weight/day and above, but not at the lowest exposure level of 1.7 mg/kg body weight/day.

Maltoni *et al.* (1981) also reported hepatic tumours in rats receiving VCM dissolved in olive oil, once daily by gavage, 4–5 days/wk for 59 wk, and then kept untreated until wk 136; namely, three hepatic angiosarcomas, one extrahepatic angiosarcoma and one hepatoma in 149 rats receiving 1 mg VCM/kg body weight/day. No liver tumours were observed in rats receiving 0.03 mg VCM/kg body weight/day. Evans *et al.* (cited by ECETOC, 1988) observed one hepatic and one subcutaneous angiosarcoma in 54 male rats receiving 25 ppm VCM in their drinking-water for up to 152 wk, a level approximately equivalent to 2 mg VCM/kg body weight/day.

Increased incidences of basophilic foci of hepatocellular alteration occurred in males of the high-dose group and in females of each of the test groups. In females, the incidence of this type of foci of cellular alteration in the liver increased with increasing dose level. Moreover, the number of rats showing foci of cellular alteration in the liver (all type of foci) was significantly higher in males of the high-dose group in females of the mid- and high-dose groups than in controls. These results suggest a relationship between the oral exposure to VCM at all dose levels and the increased occurrence of foci of cellular alteration in the liver of female rats. The incidence of basophilic foci of hepatocellular alteration in females of the control group (9%) was clearly lower than in female controls used in the previous long-term oral rat study with VCM (18%; Feron *et al.*, 1981). Incidences of 12–17% were also found in females of control groups in three other long-term rat studies carried out at our Institute more or less simultaneously with the previous VCM study. These data cast doubt on the toxicological significance of the slight increase in incidence of this type of foci in the low- and mid-dose groups in the present study. However, the incidence of basophilic foci in females of control groups of four other long-term rat studies performed at our Institute, during the same period as the present VCM study, varied from 0–8%. Compared with these values, an incidence of 9% is not unusually low, and thus it supports the suggestion that the feeding of VCM at levels of 0.014 or 0.13 mg/kg body weight/day resulted in more basophilic foci of cellular alteration in the liver of females. At these levels no hepatic neoplasms or VCM-related tumours at other sites were observed, justifying the conclusion that 0.13 mg VCM/kg body weight/day was a no-adverse-effect level with respect to the occurrence of tumours.

A directive of the European Community (EEC, 1978) imposes a maximum permitted limit of 1 ppm residual VCM in finished plastic materials or articles intended for use in contact with foodstuffs. The same

directive stipulates that any migration of VCM to food must not produce a concentration in the food greater than 10 ppb, which is close to the detection limit of VCM in foodstuffs. Calculations based on a survey of residual levels of VCM in PVC bottles, films and food in the period of 1974–1977, suggest that the human intake of VCM from the average diet was less than 0.1 $\mu\text{g/person/day}$ (Crosby, 1982; MAFF, 1978). More recent data suggest maximum average daily intakes of 0.02 $\mu\text{g/person/day}$ in the UK (MAFF, 1984) and 0.025 $\mu\text{g/person/day}$ in the USA (FDA, 1986). Extrapolation of the results of the present rat study to humans using a linear non-threshold extrapolation model, and taking into account an acceptable cancer risk from oral VCM exposure for humans of 10^{-6} , leads to an acceptable oral intake of 0.4 $\mu\text{g VCM/person/day}$. Presently, the estimated maximum oral daily intake is only 0.025 $\mu\text{g VCM/person}$, and the estimated cancer risk is 16 times lower. Moreover, the extrapolation model used is a conservative and prudent one, which implies that the actual cancer risk might be (considerably) lower than that calculated by means of the linear model. It may be emphasized that, on the basis of the results described in the present paper and the estimated intake of 0.025 $\mu\text{g/person/day}$, the FDA (1986) calculated an individual lifetime risk of below 10^{-7} , and stated that the linear proportional model used and the numerous conservatisms in the exposure estimates exaggerate the risk (FDA, 1986). Comparison of the results of an epidemiological study of vinyl chloride workers with the predicted cancer risk in a cohort of workers (based on the results of VCM studies in rats) indicates that humans are less sensitive to the carcinogenic action of VCM than are rats (Gehring *et al.*, 1979). More recent cancer risk assessments of vinyl chloride using both rat and human data suggest similar sensitivity of both species (Chen and Blancato, 1989; Swaen *et al.*, 1987). These data and considerations allow us to conclude that the cancer risk of oral daily intakes of 0.02 or even 0.1 $\mu\text{g VCM/person/day}$ is small enough to be practically negligible. Indeed, such intakes can be considered virtually safe.

Conclusions

This study showed that the ingestion of VCM at a level of 1.3 mg/kg body weight/day induces neoplastic and non-neoplastic changes in the livers of male and female rats, and that a level of 0.13 mg/kg body weight/day may result in an increased incidence of basophilic foci of cellular alteration in the livers of female but not of male rats. The no-observed-adverse-effect level with respect to the induction of tumours in rats appeared to be 0.13 mg VCM/kg body weight/day. The cancer risk of oral daily intakes of 0.02 or 0.1 $\mu\text{g VCM/person/day}$ is estimated to be negligibly small.

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