

## OBSERVATIONS ON THE ORAL ADMINISTRATION AND TOXICITY OF VINYL CHLORIDE IN RATS\*

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**Abstract**—Various possibilities were studied for administering vinyl chloride monomer (VCM) orally to rats. Upon storage, solutions of VCM in soya-bean oil appeared to be stable with respect to their VCM content and the fatty acid composition of the oil. In addition no oligomers of vinyl chloride or reaction products of VCM with components of the oil were detected. Stomach intubation of such solutions was considered an acceptable method of oral administration of VCM to rats in short-term toxicity studies. Within a period of 4 hr following intragastric intubation of VCM in soya-bean oil (300 mg VCM/kg body weight), over 92% of the VCM administered was recovered from the gases excreted (mainly exhaled?) by the animals. Eructation did not appear to be involved in the excretion of VCM.

VCM dissolved in soya-bean oil was administered by gavage to male and female rats at levels of 0 (controls), 30, 100 and 300 mg/kg body weight, once daily on 6 days/wk for a period of 13 wk. Several haematological, biochemical and organ weight values differed to a statistically significant degree from those of the controls, but these differences were considered to have only minor, if any, toxicological significance. A slight increase in liver-to-body weight ratio occurred in males and females on the highest dose level. This increase was not accompanied by liver damage, as was evident from histological examination, enzyme histochemistry and electron microscopy. The no-effect level in this 90-day study was conservatively placed at 30 mg VCM/kg body weight, but was probably higher since the effects occurring at 100 and 300 mg/kg body weight were of doubtful toxicological significance.

Indications were obtained that the feeding of rats on diets containing polyvinyl chloride (PVC) powder with a high VCM content is a more practical method for the long-term oral exposure of rats to VCM than is stomach intubation of VCM in oil. VCM was almost completely released from PVC powder during passage through the digestive tract.

### INTRODUCTION

Vinyl chloride monomer (VCM) is used in the manufacture of the resin, polyvinyl chloride (PVC). Industrial exposure to VCM has been associated with several disorders, including acro-osteolysis (Dinman, Cook, Whitehouse, Magnuson & Ditchek, 1971; Harris & Adams, 1967; Lange, Jühe, Stein & Veltman, 1974; Markowitz, McDonald, Fethiere & Kerzner, 1972) and non-malignant liver diseases (Kramer & Mutchler, 1972; Marsteller, Leibach, Müller, Jühe, Lange, Rohner & Veltman, 1973; Suciu, Drejman & Valaskai, 1967) and, only recently, also with angiosarcoma of the liver (Block, 1974; Creech & Johnson, 1974; Lee & Harry, 1974) and tumours of the brain and lungs (Monson, Peters & Johnson, 1974). Degenerative changes in the liver, kidneys, bone and brain were detected in rats and rabbits following exposure to VCM inhalation (Basalae, Vazin & Kochetkov, 1972; Jaeger, Reynolds, Conolly, Moslen, Szabo & Murphy, 1974; Torkelson, Oyen & Rowe, 1961; Viola, 1970). Moreover, VCM inhalation was found to induce tumours in both rats and mice (Maltoni & Lefemine, 1974; Viola, Bigotti & Caputo, 1971).

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Certain formulations of PVC are used widely as food-packaging materials. Residual VCM present in the extruded polymeric product was shown to be liable to migration into PVC-packed foods and drinks, especially distilled spirits (Randolph, 1973). Since no data on the oral toxicity of VCM appeared to be available, studies were initiated to examine the sub-acute toxic properties of this compound when administered orally to rats. Oral administration is greatly hampered by the fact that VCM is a gas at room temperature (b.p. c. -13.8°C) and therefore preliminary experiments were carried out to find an acceptable way of administering the compound orally to rats.

Administration in the drinking-water was considered in this respect but was rejected because of the rather poor solubility of VCM in water (Hardie, 1964) and, more significantly, because of the difficulties to be expected in handling the solutions and in estimating the quantities of VCM actually ingested by the animals.

As VCM is lipophilic, we investigated the possibility of administering VCM as a solution in edible oil either by gastric intubation or by incorporation into the diet. Another possibility studied was the addition to the diet of a PVC powder containing an unusually high concentration of VCM.

The present report describes tentative experiments on the oral administration of VCM and presents some observations on the fate of VCM in rats. In addition, the results are given of a sub-acute toxicity

study in rats given VCM dissolved in soya-bean oil by gavage for 13 wk.

#### EXPERIMENTAL

**Materials.** VCM, obtained from Akzo Zout Chemie, Rotterdam, The Netherlands, in pressurized stainless-steel cylinders, had the following specification:  $M = 62.50$ ; m.p. =  $-153.8^{\circ}\text{C}$ ; b.p. =  $-13.37^{\circ}\text{C}$ ; density =  $0.9106$ ;  $n_D = 1.3700$ . PVC powder, specially prepared to contain approximately 1800 ppm VCM, was supplied by Shell Nederland Chemie, Pernis, The Netherlands, in closed steel barrels.

**Animals and diets.** Male and female Wistar-derived rats from the Institute's colony were used. The Institute's stock diet and tap-water were offered *ad lib*.

**Solutions of VCM in soya-bean oil.** These were prepared either by bubbling VCM gas through the oil or by injecting liquid VCM into the oil. The stability of the solutions with regard to their VCM content was tested in storage experiments under various conditions either by weighing the vessels containing the solutions or by gas chromatography. For each gas-chromatographic determination, 1 g VCM-containing oil was mixed with 10 ml *N,N*-dimethylacetamide containing toluene as an internal standard. The mixture (1  $\mu\text{l}$ ) was injected on a glass column (length 2 m, i.d. 3 mm) filled with 10% DC 200 on Gas Chrom Q, 100–200 mesh. The temperature was  $90^{\circ}\text{C}$ .

The VCM solutions were also examined for polymerization products of VCM and for reaction products of VCM with unsaturated fatty acid moieties of the oil. The presence of oligomers of VCM was checked by injecting 1  $\mu\text{l}$  of a mixture of 1 ml oil and 10 ml *p*-xylene or *N,N*-dimethylacetamide directly on to two different gas chromatographic columns and comparing the chromatograms with those obtained from pure soya-bean oil diluted and chromatographed in the same way. The column used for the oil diluted with *p*-xylene was: length 2 m, i.d. 3 mm, filled with Porapak Q, 80–100 mesh, temperature  $230^{\circ}\text{C}$ . For the oil diluted with *N,N*-dimethylacetamide the DC 200 column mentioned above was used.

The fatty acid composition of the solutions in soya-bean oil was determined according to the IUPAC (Oils and Fat Section) methods 2D19 (preparation of methyl esters) and 2D25 (gas chromatography of fatty methyl esters).

**VCM in the diet.** The possibility of administering VCM to rats in the diet was studied by mixing the diet with a solution of VCM in soya-bean oil, with liquid VCM (in a closed vessel) or with PVC powder containing a high level of VCM\*. PVC powder was used either as obtained from Shell (containing about 1800 ppm VCM) or after raising its VCM content to approximately 4000 ppm by mixing the powder with a calculated amount of liquid VCM in a closed

steel barrel. The stability of the diets with respect to their VCM content was examined by storing the diets in open glass vessels at room temperature and determining their VCM content at regular intervals by gas chromatography, in the first two cases by the method described above and in the case of the diet incorporated PVC powder by mixing 5 g of the diet with 20 ml tetrahydrofuran, precipitating the PVC by adding 10 ml water, and injecting 1  $\mu\text{l}$  of the supernatant on to a glass gas-chromatographic column (length 2 m, i.d. 3 mm) filled with Porapak Q 80–100 mesh (column temperature,  $230^{\circ}\text{C}$ , carrier 50 ml nitrogen/min, detection by FID).

**Fate of VCM in oil administered orally or ip to rats.** To study the fate of VCM after oral or ip administration to rats, four separate experiments were carried out.

(1) 1 ml of a 7.2% solution of VCM in soya-bean oil was given by gavage to each of two rats (body weight 240 g, dose level 300 mg/kg), which were placed immediately after treatment in a glass exposure cylinder normally used for inhalation toxicity studies. The cylinder was connected with a trapping system consisting of a drying tube, a trapping device containing diethyl ether and an air pump with a capacity of 1 litre/7.5 min. The trapping device was cooled in a Dewar flask containing a mixture of alcohol and dry ice. Every 7.5 min the trap was replaced by a fresh one and each time the amount of VCM in the ether was determined gas chromatographically.

(2) The experiment described under (1) was repeated with two other rats, in each of which the oesophagus was ligated under anaesthesia immediately after intubation of the VCM solution.

(3) Two rats were each injected ip with 1 ml of the VCM solution in oil.

(4) The VCM content was determined in blood obtained at intervals from the tip of the tail of one rat intubated intragastrically with 1 ml of a 10% VCM solution in soya-bean oil. For the VCM determination, 0.1 ml blood was extracted with 1 ml ether, and 5  $\mu\text{l}$  ether extract was injected on a gas-chromatographic column (glass, length 2 m, i.d. 3 mm) filled with Porapak Q 80–100 mesh (column temperature,  $230^{\circ}\text{C}$ , carrier 50 ml nitrogen/min, detection by FID).

**Subacute toxicity study of VCM administered in oil by gavage to rats.** Solutions of 0, 1.0, 3.3 and 10% VCM in soya-bean oil were administered by stomach tube to groups of 15 male and 15 female weanling rats. The mean initial body weights were 44 g for both males and females. The rats in the various groups received approximate doses of VCM of 0, 30, 100 or 300 mg/kg body weight daily on 6 days/week for a period of 13 wk. The doses were adapted to the mean body weights once every week. The animals were housed in wire-screen cages in groups of three in an air-conditioned inhalation chamber (temperature  $22\text{--}24^{\circ}\text{C}$ , relative humidity 60%).

Individual body weights were recorded weekly. The food intake of each group was measured in wk 1–4 and in wk 11 and 12. Haematological indices (blood clotting time, haemoglobin content, packed cell vol-

ume and counts of total leucocytes), sugar, urea, nitro, albumin and activity aminase (GOT), glu and alkaline phosphatase (specific in ten males and females of the control group) were examined. In wk 13, urine examined for glucose, protein, microscopic constituents, urine samples from each group.

In wk 14 all rats were examined for gross spleen, brain, gonads, were weighed. Same organs were preserved for microscopic examination. High-dose Paraffin sections were prepared from lungs, salivary glands, muscle, axillary and mammary glands, pancreas, with bone marrow, mammary glands, oesophagus, duodenum, given the other dosages examined microscopically.

The following histological sections were carried out: specimens (frozen in males and five females) phosphatase (Gomori, 1953; Anderson, 1963; Glycerol phosphatase, adenosine phosphatase (Wach).

For electron microscopy (1 mm<sup>3</sup>) from one male and two females were fixed in 1% osmium tetroxide, sodium cacodylate, cubes were dehydrated in Epon resin. Section toluidine blue for were cut on an LKB sections were stained with citrate and examined microscopically.

#### Stability of VCM

When solutions stored in open vials, content diminished

\*By Dr. R. Kroes of National Institute for Environmental Health Research, The Netherlands.

†The ultrastructural studies by Mr. R. Handy, Industrial Biologist, England.

\*Attempts were made unsuccessfully to encapsulate VCM in malto-dextrin (type Snowflake 01902, Corn Products Company, 'Sas van Gent' B.V., Nederland), by freeze- or oven-drying of emulsions of soya-bean oil containing 10% VCM in an aqueous solution of malto-dextrin and sodium caseinate (emulsifier).

ume and counts of erythrocytes and total and differential leucocytes) and biochemical blood values (sugar, urea nitrogen, total serum protein, serum albumin and activities of glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT) and alkaline phosphatase) were recorded terminally in ten males and ten females of each group. Kidney function (specific gravity and activity of GOT in the urine) was examined in wk 13 in ten males and ten females of the control and top-dose group. Also in wk 13, urine examinations, including appearance, pH, glucose, protein, occult blood, ketones and microscopic constituents, were conducted upon pooled urine samples from ten males and ten females of each group.

In wk 14 all rats were killed by decapitation and examined for gross changes. The heart, kidneys, liver, spleen, brain, gonads, thymus, thyroid and adrenals were weighed. Samples of these and a range of other organs were preserved in 10% buffered formalin. Detailed microscopic examination was performed on all rats of the high-dose group and on the controls. Paraffin sections stained with haematoxylin-eosin were prepared from the weighed organs and from the lungs, salivary glands, trachea, thoracic aorta, skeletal muscle, axillary and mesenteric lymph nodes, ceruminous glands, pancreas, urinary bladder, sternum with bone marrow, prostate, epididymis, uterus, mammary glands, oesophagus, fore and glandular stomach, duodenum, ileum, caecum and colon. In rats given the other dose levels, only the liver was examined microscopically.

The following histochemical enzyme determinations were carried out\* on cryostat sections of liver specimens (frozen in isopentane at  $-70^{\circ}\text{C}$ ) from five males and five females of each group: alkaline phosphatase (Gomori, 1939), acid phosphatase (Barka & Anderson, 1963; Gomori, 1939) and adenosine monophosphatase, adenosine triphosphatase and glucose-6-phosphatase (Wachstein & Meisel, 1957).

For electron microscopy, small pieces of liver (1 mm<sup>3</sup>) from one male and one female control rat and from two males and two females from each test group were fixed in 1% osmium tetroxide buffered with 0.1 M-sodium cacodylate (pH 7.4) for 2 hr at  $4^{\circ}\text{C}$ . The cubes were dehydrated in alcohol and embedded in Epon resin. Sections (1  $\mu\text{m}$ ) were cut and stained with toluidine blue for light microscopy. Selected areas were cut on an LKB ultramicrotome and ultrathin sections were stained with uranyl acetate and lead citrate and examined in a AEI EM6B electron microscope.

## RESULTS

### Stability of VCM solutions in soya-bean oil

When solutions of VCM in soya-bean oil were stored in open vials at room temperature, their VCM content diminished at a fairly low rate. For example,

Table 1. Fatty acid composition of pure and VCM-containing soya-bean oils after storage at  $37^{\circ}\text{C}$  for a period of 8 days

| Fatty acid | Fatty acid content (%) of |                        |
|------------|---------------------------|------------------------|
|            | Pure oil                  | Oil containing 10% VCM |
| Myristic   | 0.2                       | 0.2                    |
| Palmitic   | 10.9                      | 11.0                   |
| Stearic    | 3.6                       | 3.8                    |
| Oleic      | 22.8                      | 22.7                   |
| Linoleic   | 55.8                      | 55.8                   |
| Linolenic  | 6.7                       | 6.6                    |

a 5.6% VCM solution had lost only 10% of its VCM after 1 hr, 16% after 4 hr and 29% after 8 hr. A 0.5% solution appeared to lose 25% of its VCM after a storage period of 24 hr, 50% after 4 days and 80% after 8 days.

Comparison of the chromatograms of a 10% VCM solution in soya-bean oil stored in a closed vial for a period of 8 days at  $37^{\circ}\text{C}$  with those of pure soya-bean oil did not reveal any evidence of the formation of vinyl chloride oligomers or other reaction products of VCM with oil compounds during the storage of the VCM solutions. Neither was the fatty acid composition of soya-bean oil significantly altered by VCM present in the oil for a prolonged period of time (Table 1).

### VCM in the diet

A large part of the VCM evaporated during the mixing of a VCM solution in soya-bean oil with the stock diet (Fig. 1) and during storage of this diet in

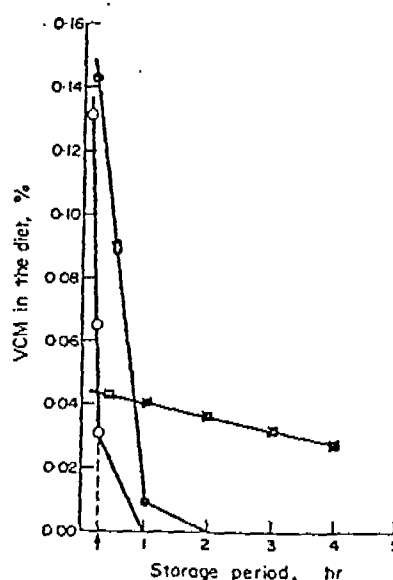


Fig. 1. Stability of VCM content of stock diet during storage at room temperature in open vessels, following mixing of the diet with soya-bean oil containing 3% VCM (9:1, w/w) in an open vessel (O), with soya-bean oil and liquid VCM (9:1:0.005, by weight) in a closed steel barrel (●) or with PVC containing 0.44% VCM (9:1, w/w) (■); ↑ indicates end of mixing period. The VCM determinations were carried out by gas-chromatography, using the method

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†The ultrastructural studies on the liver were carried out by Mr. R. Hendy and Mr. M. Wright of the British Industrial Biological Research Association, Carshalton, England.

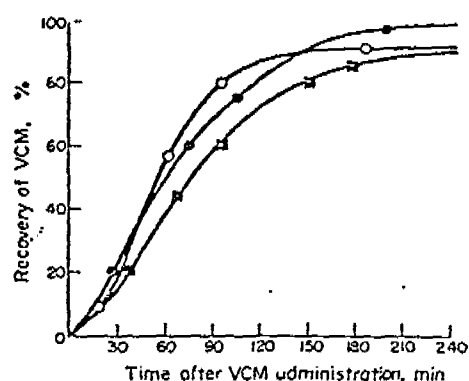


Fig. 2. Recovery of VCM from the atmosphere surrounding rats following administration of a dose of 300 mg VCM/kg body weight (as a 7.2% VCM solution in soya-bean oil) either by stomach tube to rats with an open (O) or ligated (■) oesophagus or by ip injection (●). A 1- $\mu$ l aliquot of the ether used for trapping the VCM in the atmosphere was injected on to a glass gas-chromatographic column (length 2 m, i.d. 3 mm) filled with Porapak Q 80-100 mesh (column temperature 230°C, carrier 50 ml nitrogen/min, detection by FID).

an open vial the VCM content decreased to an undetectable level in a period of 1 hr. The rate of decrease of the VCM content of the diet was slightly lower following mixing of the diet with liquid VCM in a closed vessel (Fig. 1).

When PVC powder was stored at room temperature in an open vial, its VCM content decreased relatively slowly. For example, an initial VCM content of 1850 ppm decreased to 750 ppm after a storage period of 6 hr and to 85 ppm after 70 hr of storage. PVC powder spread in a very thin layer on plates in a drying oven at 50°C lost its VCM completely (VCM content less than 0.3 ppm) within a period of 2-4 hr but when larger amounts of PVC (e.g. 50 kg)

were to be freed from VCM, it appeared necessary to keep the powder (in layers of 4-6 cm thick) in a vacuum oven at 60°C for a period of at least 60 hr.

A 9:1 mixture of stock diet and PVC powder containing a high level of VCM (e.g. 4400 ppm) lost VCM at a fairly slow rate when stored at room temperature in animal feeders. Such a diet still contained approximately 60% of the initial amount of VCM after a storage period of 4 hr (Fig. 1). The stability of the diet as to its VCM content was slightly improved (by roughly 10%) when the PVC powder was mixed with a 1% aqueous solution of guar gum or pregelatinized starch before addition to the diet.

When rats were fed a diet containing 10% PVC powder with a VCM content of 1850 ppm for a period of 2 days (receiving a fresh portion of the diet at 17:00 hr on days 1 and 2 and being killed the next morning at 09:00 hr), the VCM level of the contents of the colon and rectum was found to be virtually nil, indicating that VCM was released almost completely from the PVC powder during its passage through the digestive tract.

#### Fate of VCM given in oil by gavage or ip injection

Within 4 hr of the oral or ip administration of VCM at a dose level of 300 mg/kg body weight over 92% of the administered VCM was recovered from the atmosphere surrounding the animals (Fig. 2). The VCM recovery curves were not significantly influenced by the way in which the compound was administered, which suggests *inter alia* that belching of VCM did not play a significant role in the excretion of the compound by rats. VCM was detectable in the tail blood only 2 min after intragastric intubation of a VCM solution in soya-bean oil. The VCM content of the blood reached its maximum (1.9  $\mu$ g/ml) about 10 min after dosing and decreased to an undetectable level in the next 40 min.

Table 2. Mean results of administering 0-300 mg VCM/kg body weight/day to rats by gavage on 6 days/wk for 13 wk, showing differences of possible toxicological significance between test and control animals

| Parameter affected                      | Values for males given VCM in doses (mg/kg) of |        |        |         | Values for females given VCM in doses (mg/kg) of |        |        |         |
|-----------------------------------------|------------------------------------------------|--------|--------|---------|--------------------------------------------------|--------|--------|---------|
|                                         | 0                                              | 30     | 100    | 300     | 0                                                | 30     | 100    | 300     |
| Total leucocytes ( $10^3/\text{mm}^3$ ) | 18.5                                           | 18.8   | 17.0   | 16.5    | 17.3                                             | 17.6   | 14.6*  | 14.8*   |
| Blood sugar (mg/100 ml)                 | 88                                             | 84     | 78**   | 77**    | 89                                               | 88     | 74***  | 79**    |
| Serum: GOT (R-Fu)                       | 228                                            | 217    | 224    | 201*    | 227                                              | 212    | 212    | 200     |
| GPT (R-Fu)                              | 53                                             | 49     | 48     | 45*     | 42                                               | 38     | 38     | 39      |
| Urinary GOT (R-Fu)                      | 30                                             | NE     | NE     | 22*     | 16                                               | NE     | NE     | 18      |
| Liver weight (g/100 g body weight)      | 3.51                                           | 3.60   | 3.74   | 3.76*   | 3.32                                             | 3.34   | 3.51   | 3.71*** |
| Adrenal weight (g/100 g body weight)    | 0.0158                                         | 0.0147 | 0.0143 | 0.0133* | 0.0247                                           | 0.0241 | 0.0239 | 0.0244  |
| Foci of hyperbasophilic hepatocytes†    | 0                                              | 0      | 1      | 1       | 0                                                | 0      | 1      | 1       |
| RER in liver cells                      | Normal                                         | NE     | NE     | Ht      | Normal                                           | NE     | NE     | Ht      |

GOT = Glutamic-oxalacetic transaminase GPT = Glutamic-pyruvic transaminase R-Fu = Reitman-Frankel units  
NE = Not examined RER = Rough endoplasmic reticulum Ht = Hypertrophic

†No. of rats affected/group.

Values are the means of at least ten rats, except for the electron-microscope observations on the liver which were made on one control and two test animals. Those marked with asterisks differ significantly (Wilcoxon's test) from those of the controls: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

#### Toxicity of VCM to rats

The gavage a soya-bean oil at did not cause any or behaviour. The total number of v cent of the blood mediate and high of serum GOT as decreased in male were no other sign al or biochemical alterations were c tients of the urine

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Since solutions of to be stable when s longed period of tim for the oral adminis in rats. However, the in the diet was rega diet was found to lo: Stomach intubation

#### Toxicity of VCM administered in oil by stomach tube to rats

The gavage administration of VCM solutions in soya-bean oil at levels up to 300 mg/kg body weight did not cause any noticeable changes in appearance or behaviour, body-weight gain or food intake. The total number of white blood cells and the sugar content of the blood were slightly decreased by the intermediate and high dose levels (Table 2). The activities of serum GOT and GPT and of urinary GOT were decreased in males given the top dose (Table 2). There were no other significant changes in the haematological or biochemical indices and no treatment-related alterations were observed in the microscopic constituents of the urine.

The relative weight of the liver in males and females showed a tendency to increase with increasing doses of VCM but the difference from the controls was statistically significant only at the highest dose level (Table 2). In addition a dose-related decrease in the relative weight of the adrenals occurred in males, the difference from the controls being statistically significant again only in the high-dose group. The other organ weights recorded were closely comparable in all groups.

Minimal histological changes in the liver, seen as one or a few foci of 40-70 hyperbasophilic hepatocytes, occurred in some rats of the intermediate and high-dose groups (Table 2). The hyperbasophilic foci were not accompanied by architectural liver changes. Randomly distributed histopathological changes unrelated to treatment included small accumulations of reticulo-endothelial cells in the liver, focal tubular nephrosis, a slight degree of nephrocalcinosis, parasites (*Trichosomoides crassicauda*) in the renal pelvis and urinary bladder, early signs of chronic respiratory disease in the lungs, granulomatous pneumonic foci, medial hypertrophy of medium-sized pulmonary arteries, 'morphological' activation of the thyroid gland, focal myocarditis, transformation of pancreatic acini into duct-like structures, 'squamous' metaplasia of the epithelium lining excretory ducts of the sublingual salivary gland and slight prostatitis.

The histochemical studies of liver enzymes failed to reveal any difference between test and control animals either in the concentrations or distribution patterns of the various enzymes examined.

Electron microscopic examination of the liver showed hypertrophy of the endoplasmic reticulum in hepatocytes of animals given the highest dose. No difference from controls was observed with respect to other liver-cell constituents, and the capillary endothelial cells and the Kupffer cells were ultrastructurally indistinguishable from those of the controls.

#### DISCUSSION

Since solutions of VCM in soya-bean oil appeared to be stable when stored in closed vials for a prolonged period of time, they were considered suitable for the oral administration of VCM in toxicity studies to rats. However, the feeding of such VCM solutions to rats was regarded as inadequate because the oil was found to lose its VCM in a very short time. Stomach intubation turned out to be an acceptable

alternative, especially as there was no experimental evidence that eructation was involved in the excretion of VCM by the rats. Moreover, since the level of VCM in the blood reached its maximum within only 10 min of gastric intubation and by far the greater part of the administered VCM was recovered from the gases excreted (mainly exhaled?) within 4 hr, it was apparent that VCM given by gavage was readily absorbed from the gastro-intestinal tract.

From the toxicity study in which VCM was administered by stomach intubation, it appeared that several haematological and biochemical indices (white blood-cell counts, blood-sugar content and activities of serum GOT and GPT and urinary GOT) were lower to a statistically significant degree in the groups given the intermediate and high doses, or just in the group given the highest dose, than in the controls. In all cases, however, the values in the test groups were within the normal range and, moreover, the values obtained for the control animals were invariably very close to the upper limit of normality. Therefore, these differences between test and control animals were considered of minor, if any, toxicological significance.

Exposures to atmospheres containing VCM have been reported to result in malignant and non-malignant liver changes in several animal species and in man (Block, 1974; Falk, Creech, Heath, Johnson & Key, 1974; Kramer & Mutchler, 1972; Lange *et al.* 1974; Makk, Creech, Whelan & Johnson, 1974; Maltoni & Lefemine, 1974; Marsteller *et al.* 1973; Torkelson *et al.* 1961; Viola *et al.* 1971). In the present experiment on the oral toxicity of VCM, a significant increase in liver-to-body weight ratio found at the highest dose level in both males and females may also have been indicative of a toxic effect of VCM on the liver. This suggestion was not clearly supported, however, by the histological liver changes observed (foci of hyperbasophilic hepatocytes), which were found only in a few rats on the two higher dose levels and which were very slight in degree and not necessarily related to treatment. In addition, the histochemical enzyme studies failed to produce evidence of any effect of VCM on the liver, while the hypertrophy of the rough endoplasmic reticulum found in hepatocytes of top-dose animals without other ultrastructural changes was probably a non-specific reaction not necessarily indicative of a toxic response. The absence of liver damage in the present study may be related to the relatively short duration of the experiment and the short daily exposure of the rats to VCM.

At the highest dose level, a statistically significant depression of the relative weights of the adrenals compared with the controls was seen in males. This decrease was due, at least in part, to the relatively heavy adrenals of the control animals (the adrenal-to-body weight ratio for rats of the strain and age used being on average 0.0138 g/100 g); it did not occur at all in females and was not accompanied by histological changes in the adrenals. The decrease, therefore, was not considered to be an effect of toxicological importance.

Though special attention was paid to the microscopic appearance of the ceruminous glands (Zymbal's glands), which are known to be target organs

of VCM administered by inhalation (Maltoni & Lefemine, 1974), no histopathological changes could be detected at this site.

From this oral toxicity study, the no-toxic-effect level may be placed at 30 mg VCM/kg body weight, but it may be higher, as the effects noticed at the 100- and 300-mg/kg dosage levels were considered of doubtful toxicological significance.

Although daily administration of VCM in oil by gavage seems to be acceptable also for long-term studies, the method has several disadvantages. These include technical problems inherent in the daily intubation of a large number of rats for a prolonged period, the daily exposure of the test animals to a very large amount of VCM for a relatively short time and the difficulty of interpreting the toxicological significance of possible gastric lesions. Observations described in the present report indicate that the feeding of rats on diets containing PVC powder with a high VCM content is a more practical way of administering the test compound orally than is stomach intubation and is, moreover, unencumbered by the above problems. It may be argued that even when rats are provided daily with a fresh portion of feed, the loss of VCM from the diet is considerable (about 80%) over a 24-hr period. This difficulty can be obviated, however, by training the rats to eat their daily portion of diet within a period of 2-4 hr, a procedure which is still a much more realistic way of administering VCM than is stomach intubation. In addition, shortening the feeding period has the advantage of reducing automatically the period during which rats are unavoidably exposed to VCM evaporating from the test diets.

Starting with PVC powder containing 4000 ppm VCM, a maximal level of PVC in the diet of 10% and a (pessimistically) estimated average 50% loss of VCM from the diet before ingestion, VCM can be administered in this way at a maximum dietary level of 200 ppm, which is equivalent to 10 mg VCM/kg body weight/day. This level is very high when compared with the likely oral daily intake by man in Europe, estimated to be less than 0.0017 mg/kg body weight (van Esch & van Logten, 1975), but is relatively low compared with the 300 mg VCM/kg body weight (given daily to rats by gavage), which merely induced effects of questionable toxicological importance. A 2-yr chronic toxicity/carcinogenicity study in rats receiving VCM in either soya-bean oil by gavage (one very high level) or in PVC powder incorporated into the diet (three much lower levels) is currently in progress.

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