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INTER-OFFICE CORRESPONDENCE

Date: May 21, 1979

From: Z. G. Bell

Location: 5 West

Subject: Lifespan Oral Toxicity Study of VCM in Rats

To: F. C. Dehn

Attached is the abstract of the VCM study conducted in West Germany. It is via the oral route. Not much new information gained from this work in my opinion. The full report is in the file if you are interested.

Zeb G. Bell, Jr.

/ta

Attachment

cc: G. J. Lazarchik
A. P. Leber
H. B. Lovejoy

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CENTRAAL INSTITUUT VOOR VOEDINGSONDERZOEK TNO

CENTRAL INSTITUTE FOR NUTRITION AND FOOD RESEARCH

Utrechtseweg 48
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REPORT NO. R 5788

Title: Life-span oral toxicity study of vinyl chloride in rats*

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At the request of: Verband kunststofferzeugende Industrie e.V., Frankfurt a.M. W. Germany

Date: October 1978

Project no.: 674/5603

Approved by: Dr. A.P. de Groot

Start of study: 13 January 1975

Termination of study: 13 October 1977

Study director: Dr. V.J. Feron

All raw data and the final report are stored in the archives of the Department of Toxicology under the reference "Verband kunststofferzeugende Industrie".

*This study was sponsored by a group of co-operating European industries including Verband Kunststofferzeugende Industrie e.V. (Federal Republic of Germany), Shell Nederland Chemie, Dutch State Mines, Akzo Zout Chemie Nederland BV and Dow Chemical Europe S.A.

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SUMMARY

1. A life-span oral toxicity study including interim kills after 26 and 52 weeks, was carried out with vinyl chloride monomer (VCM) in Wistar rats. VCM was administered either by incorporating PVC-powder with a high VCM content into the diet or by gastric intubation of a 10 per cent VCM solution in soya bean oil.
The diets containing PVC -powder were provided daily for a period of four consecutive hours, whereas food was withdrawn during the other 20 hours. The use of this way of oral VCM administration resulted in the following oral exposure levels: 0 (control group), 1.7, 5.0 and 14.1 mg VCM/kg body weight/day.
Since a) the feeding of PVC-powder in the diet did not allow the use of dietary VCM levels much higher than 14.1 mg/kg body weight/day, and b) it was deemed desirable to include at least one dose-group that would show overt signs of VCM toxicity, one group of rats was treated with VCM in soya bean oil by gavage at a reasonably high dose, viz. 300 mg/kg body weight on five days a week.
Observations were made of general appearance, mortality, growth, food intake, hematology, biochemistry of the blood, urine and liver, organ weights, gross and microscopic pathology and electron microscopy of the liver.

2. Growth-retardation was seen only in animals of the 300 mg/kg group. Death-rate increased with increasing VCM levels. At the lowest dose level mortality was slightly increased only in females towards the end of the experimental period.

3. Blood-clotting time was slightly shortened in animals of the 14.1 and 300 mg/kg groups. There was also a slight increase in α-feto-protein content of the blood serum in these groups.

4. Slight liver-enlargement and an increased haematopoietic-activity in the spleen occurred in males and females of the two highest dose groups.

S U M M A R Y (continued)

5. A variety of neoplastic and non-neoplastic VCM-related liver lesions were found in each of the test groups. Hepatic angiosarcomas occurred at levels of 5.0 mg/kg and higher. Hepatocellular tumours (neoplastic nodules and carcinomas) were found in all dose-groups. Their incidence was high in both sexes of the 14.1 mg/kg group and in females of the 1.7 and 5.0 mg/kg groups, low in males of the 5.0 mg/kg group, and marginal in males of the 1.7 mg/kg group.

6. Pulmonary angiosarcomas (mainly metastases) and a few extrahepatic intra-abdominal angiosarcomas were observed at levels of 5.0 mg/kg and higher.

7. A few Zymbal gland tumours occurred at the 5.0 amd 300 mg/kg levels.

8. There was some indication that VCM may enhance the development of both intra-abdominal mesotheliomas and adenocarcinomas of the mammary glands.

9. Mitochondrial alterations were the earliest and most characteristic VCM-induced ultrastructural changes in hepatocytes.

10. The ultrastructure of angiosarcoma cells in the liver was suggestive of the tumour cells being derived from sinusoidal endothelium. Clear fibrosis was not seen to precede angiosarcoma-formation in the liver.

11. Main conclusions were:

- VCM is a carcinogen in rats when administered by the oral route;
- the tumour response of the liver of the rat to oral intake of VCM seems to shift from almost exclusively angiosarcoma at very high levels to exclusively hepatocellular tumours at low levels;
- the "no-toxic effect level" was lower than 1.7 mg VCM/kg body weight/day under the rigorous conditions of continuous exposure (24 hours a day) resulting from the continuous release of VCM from PVC-powder in the gastro-intestinal tract.

Life-span oral toxicity study of vinyl chloride in rats

1. INTRODUCTION

Industrial exposure to vinyl chloride monomer (VCM) has been associated with several disorders such as acro-osteolysis, non-malignant liver disease, angiosarcoma and carcinoma of the liver, and tumours of the brain and lungs (Anonymus, 1976; Berk et al, 1976; Delorme & Makk, 1976; Falk and Maxweiler, 1976; Haley, 1975; Jühe et al, 1973; Makk et al, 1976; Monson et al, 1974; Thomas and Popper, 1975; Vale et al, 1976). In addition, various types of malignant tumours 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 (Bartsch and Montesano, 1975; Basalaev et al, 1972; Feron et al, 1977; Jaeger et al, 1974; Keplinger et al, 1975; Lee et al, 1978; Maltoni and Lefemine, 1974; 1975; Maltoni et al, 1974; Müller et al, 1975; Suzuki , 1978; Torkelson et al, 1961; Viola et al, 1971; Williamson, 1976; Winell et al, 1976).

Residual VCM present in extruded polyvinyl chloride (PVC) has been shown to be liable to migration into PVC-packed foods and drinks (Daniels and Proctor, 1975; Fuchs et al, 1975; Potter, 1976; Randolph, 1973; Williams and Miles, 1975). There is still only a small amount of data on the oral toxicity of VCM. In a 13-week toxicity study of VCM conducted in this Institute 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 for six days a week. 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. In addition, a slight increase in liver-to-body weight ratio occurred at the highest dose level. This increase was not accompanied by morphological liver changes. The no-effect-level in this 90-day study was conservatively placed at 30 mg/kg body weight, but was probably higher since the effects occurring at 100 and 300 mg/kg body weight were of doubtful toxicological significance (Feron et al, 1975). From preliminary observations it appeared that an alternative and 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 life-span oral toxicity study of VCM in rats, PVC-powder containing a high level of VCM was incorporated in the

diet at levels resulting in planned daily intakes of 1, 3 or 10 mg VCM/kg body weight. This method of feeding PVC-powder in the diet does not allow the use of dietary VCM levels which are much higher than the top-dose level of 10 mg/kg body weight/day which was chosen for the present experiment. However, this level is high when compared with the maximum likely oral daily intake by man, which has been estimated to be 1.7 µg/kg body weight (Van Esch and Van Logten, 1975), and even very high in comparison with a more recent estimate of the maximum likely intake by man, viz. 0.0017 µg/kg body weight/day or 0.1 µg/human/day (Anonymus, 1978). On the other hand the level of 10 mg/kg body weight/day is low in comparison with the dose of 300 mg VCM/kg body weight (given daily by gavage for six days a week), which was not an obvious toxic-effect-level in the 13-week study mentioned above (Feron et al, 1975). Since the dose levels to be used in a chronic toxicity study should include at least one effect-level, it was deemed desirable to include one group of rats in the present long-term study receiving VCM in soya bean oil by gavage at a reasonably high dose, viz. 300 mg/kg body weight, five days a week, despite the disadvantages of gastric intubation.

2. MATERIAL AND METHODS

2.1. Materials

Vinyl chloride monomer (VCM), from Akzo Zout Chemie, Rotterdam, The Netherlands. Physical chemical constants:
Mw = 62.50; m.p. = -153.8°C; b.p. = -13.37°C; density = 0.9106; n_D = 1.3700. The product (colourless, clear, free of suspended matter) was obtained in pressurized stainless-steel cylinders, and had the following standard specification, which was provided by the supplier:
Vinyl chloride monomer ≥ 99.97 wt % min.; acetylene ≤ 2 µl/l (gas); mono-vinylacetylene ≤ 15 µl/l (gas); 1,3-butandiene ≤ 10 µl/l (gas); methyl chloride ≤ 75 µl/l (gas); ethyl chloride ≤ 50 µl/l (gas); chloroprene ≤ 1 µl/l (gas); 1,1-dichloroethane ≤ 1 µl/l (gas); 1,2-dichloroethane ≤ 20 µl/l (gas); acetaldehyde ≤ 5 mg/kg; hydrochloric acid ≤ 1 mg/kg; iron ≤ 0.5 mg/kg; water ≤ 100 mg/kg; evaporation residue ≤ 10 mg/kg.

PVC-powder, commercial name Carina S 65-02, was supplied by Shell Nederland Chemie, Pernis, The Netherlands, in closed steel barrels. The particle size distribution (by weight), provided by the supplier, was: 0.1 % max. > 300 µm; 4 % max. > 200 µm; 90 % max. > 88 µm; 95 % max. > 40 µm.

Three different batches were used : 180 kg (VCM content 1800 ppm) received on 10 July 1974, 1000 kg (VCM content 1500 ppm) received on 14 April 1975, and 500 kg (VCM content 3 ppm) received on 13 January 1977.

The VCM content of the PVC-powder was raised to approximately 4000 ppm by mixing the powder with a calculated amount of the liquid VCM in a closed steel barrel, containing approximately 50 kg PVC.
This PVC-powder was stored in tightly closed steel containers in a refrigerator at 4°C until a few minutes before mixing with the diet.
Part of the PVC-powder as obtained from Shell was freed from VCM by keeping the powder in layers of 4 to 6 cm thick in a vacuum oven at 60°C for a period of 3 to 4 days. After this treatment the VCM content of the PVC-powder was 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, 1973). The solution was stored at 4°C for a period of, at most, 4 weeks.

2.2. Dosage levels of VCM

2.2.1. PVC-containing diets

2.2.1.1. Preparation and administration of the diets

Each of the diets contained 10 % PVC powder as specified below:

groups	% PVC-powder in diet	
	PVC-powder containing approximately 4000 ppm VCM	PVC-powder without VCM[1])
control group	0	10
low dose group	1	9
mid dose group	3	7
high dose group	10	0

[1]) PVC-powder freed from VCM. The VCM-content was lower than 0.3 ppm

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 offering the diets to the rats. The composition of the stock diet is presented in table 1. The levels of nutrients and contaminants in stock diet are determined periodically (see annex). The diets were available to the rats each day for a period of four hours (generally between 10.00 a.m. and 02.00 p.m), in such quantities that the animals did not consume all the feed. At the end of the four-hour feeding-period the feeders were removed from the cages and the remainder of the diets was destroyed.

The rats had constant access to bottled tap water.

2.2.1.2. Actual oral exposure levels of VCM

When PVC-powder containing, say, 4000 ppm VCM is incorporated in the diet of rats at levels of 1, 3 and 10 percent, and an assumed average loss of 50 per cent of VCM from the diet before ingestion is taken into account, the dietary VCM levels would be 20, 60 and 200 ppm respectively. This corresponds with exposure levels of approximately 1, 3 and 10 mg VCM/kg body weight/day. The actual oral exposure levels will very probably be somewhat lower than the design levels, because it is unlikely that the VCM present in the powder is released completely during the passage of the powder through the gastro-intestinal tract.

In order to be able to calculate the actual oral exposure levels of VCM, the following information is needed: (a) the amount of VCM evaporating from the diets during the four-hour feeding-period; (b) the speed with which the animals consume 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 hour, 2 hours and 4 hours. The diet samples to be analyzed were taken at random from the feeders in the cages, without homogenizing the diets in the feeders. In this way samples of each of the test diets were taken on 11 or 12 different days, and were analyzed for their VCM-content. The analyses were carried out by means of gas-liquid chromatography according to a method described in a previous report (Feron et al, 1975). The average VCM-contents of the various diets, found at the different points of time, are graphically depicted in Fig. 1, on page 79.

The eating-speed was determined by measuring the amount of residual feed in the feeders after periods of 1 hour, 2 hours and 4 hours. This was performed for each of the diets on four different days. The number of rats involved in these determinations varied from 10 to 40/sex/group. Since no appreciable variations were encountered in the rate of food consumption of the animals in the various groups, an average rate of food consumption was calculated for both males and females (Fig. 1).

The VCM intake (mg/kg body weight/day) 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 four-hour feeding-

period for male and female rats of each group. Both graphs were 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 was obtained by adding the VCM intakes during the first and second hour, and the last two hours of the four-hour feeding period. The VCM intake was subsequently expressed as 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. It 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 the amount of VCM excreted in the feces, freshly produced feces from three to five males and three to five females of each of the test groups were collected at 9.00 a.m. (one hour before the start of the feeding period), 2.00 p.m. (at the end of the feeding period), 6.00 p.m. (4 hours after termination of the feeding period) and 11.00 p.m. (9 hours after termination of the feeding period). Fresh feces samples were obtained by squeezing the lower part of the rat's abdomen. The droppings were weighed, submerged in 10 ml ethylacetate, and stored at 4°C in a closed vessel until analysis of the supernatant liquid by gas chromatography. This procedure was repeated twice during the study, for each of the groups and for each of the sexes, and each time using different rats.

No appreciable differences in VCM content of the feces were found within a particular test group, between the various points of time at which the droppings were collected, although the optimum value was invariably obtained from feces which were collected 9 hours after termination of the feeding-period (11.00 p.m.). Nor were there appreciable differences in VCM content of the feces between males and females.

The average amount of VCM found in the feces, 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 regularly determined. During the course of the study some twenty determinations per

dosage level were carried out[1].
The results of these VCM-determinations formed the basis for calculating the average VCM-contents of the various diets, which appeared to be 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 respectively (see also table 2).

The actual VCM-levels were clearly higher than the designed levels of 1, 3 and 10 mg/kg body weight/day. The differences are due to the fact that the loss by evaporation of VCM from the diets before ingestion was found to be much smaller, viz. 20 per cent, than the assumed loss of 50 per cent.

2.3. 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 once a day, five days a week, for a period of 83 weeks, 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 (table 1) and bottled tap water ad libitum.

For practical reasons a comparable control group receiving soya bean oil without VCM was not included in the study.

2.4. Animals and housing

360 male and 360 female newly weaned, albino Wistar rats (Cpb:WU; Wistar random), which were obtained from the SPF colony of the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, Netherlands, were allocated randomly over 5 groups of males and 5 groups of females in such a way that the mean body weights were virtually the same. Three of the groups, viz. the control group and the two highest dose groups, each consisted of 80 males and 80 females. The two lowest dose groups each comprised 60 males and 60 females.

During a period of 5 days prior to the start of the experiment, the rats which were to be fed the PVC-containing diets received stock diet without PVC for four to six hours each day. This was done to give

1) Daily determinations were not considered necessary because a) the VCM-content of the PVC-powder stored at 4°C in a closed barrel was found to remain constant; and b) the VCM-content of the PVC-powder used appeared to correspond very well with the VCM-content of the various diets.

the animals some chance to adapt to the daily feeding-period of "exactly" four hours which was to be used during the entire test period.

The study was started on 13 January 1975 and terminated when about three fourths of the controls was dead. This point of time was reached for males in week 135 (date of termination 12 August 1977) and for females in week 144 (date of termination 13 October 1977).

At the start of the study the rats were 5 weeks old, and the average body weights of the animals which were to be fed the PVC diets were: for males 92±0.5 g (range 71-115 g), and for females 86±0.5 g (range 61-106 g). The average initial body weights of males and females which were to be treated with VCM in oil by gavage (and thus not adapted to a reduced daily feeding-period) were 113±1.0 g (range 94-132 g) and 96±0.9 g (range 74-118 g) respectively.

The rats receiving PVC-powder in their diet were housed under conventional conditions, in groups of five in a suspended type of stainless steel cage with a wire screen-bottom (17x44x32 cm), in a well-ventilated room maintained at a temperature of 24±1°C. The rats given VCM by gavage were housed in groups of two in suspended, tinned wire-screen cages (19x19x32 cm) in a well-ventilated cabinet (1.45x2.15x1.20 m) maintained at a temperature od 25-28°C. Animals which were in bad condition were housed individually in separate cages until they died or were killed because their condition was so bad that they were not expected to last out the night, or, when observed on Friday, the weekend.

In order to get an idea of the VCM concentration in the atmosphere of the room containing the rats fed the PVC diets, samples of the air taken at different sites in the room (i.a. immediately above one of the feeders containing the diet with the highest level of VCM-containing PVC-powder) were analyzed for the presence of VCM. In no case could VCM be detected, indicating that the VCM concentration in the atmosphere was lower than the detection limit of 0.2 ppm.

Attempts were made to determine the concentration of PVC-particles in the atmosphere of the animal room. Air was collected during a period of 24 hours. The total dust concentration in the atmosphere of the room was found to be 0.1 mg/m^3 of air.

2.5. Conduct of the experiment

2.5.1. Body weights and food consumption

The rats were individually weighed, initially, and at weeks 1, 2, 4, 6, 8, 10 and 12, and at 4-week intervals thereafter.

Food consumption of 20 rats/sex/group was measured during weeks 1-2, 10-11, 24-25, 36-37, 60-61, 72-73 and 84-85.

2.5.2. Haematology

Blood samples were collected from the tip of the tail of 10 rats/sex/group in week 13, 26, 52, 78 and 94. All samples were examined for haemoglobin concentration (Hb) by the cyanmethaemoglobin method of Van Kampen and Zijlstra (1961); packed cell volume as micro-haematocrit; thrombocyte and red and white blood cell counts by Coulter Counter; and differential white cell count by direct visual count of smears after Pappenheim staining according to Gorter and De Graaff (1955).

2.5.3. Clinical chemistry

Fasting blood glucose and blood urea nitrogen (BUN) were determined in weeks 13, 26, 52 and 106 using the Technicon AutoAnalyzer method N-9a for glucose, and the automated phenazone/diacetyl monoxime technique of Ceriotti and Spandrio (1965) for urea. The analyses were conducted upon blood from the tip of the tail of 10 rats/sex/group after the animals had been fasted overnight.

In the course of weeks 13, 26, 52 and 106 samples of blood were collected from the orbital sinus of 10 rats/sex/group. The following measurements were made in the serum after centrifugation at 3000 rpm for 20 minutes: alkaline phosphatase (SAP), by the method of Bessey et al (1946), using a Technicon AutoAnalyzer; glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT), according to the method of Reitman and Frankel (1957), using a Technicon Auto-Analyzer; total protein (TSP), by biuret reaction; albumin according to the method of De Leeuw-Israel et al (1967); serum protein pattern by the agar-gel electrophoretic method on microscope slides of Wieme (1965), staining with nigrosin and quantitative evaluation of the electropherograms by transmission densitometry of the strips.

2.5.4. Urinalyses

Individual urine samples were collected from 10 rats sex/group in weeks 13, 26, 52, 78 and 94, i.e. during the last 16 hours of a 24-hour period of deprivation of food and water. The following measurements were made: volume (in calibrated tubes); specific gravity (by an Abbe-type refractometer); urine acid according to Gorter and De Graaff (1955), glutamic-oxalacetic transaminase activity (SGOT), according to the method of Reitman and Frankel (1957), using reagents from Baker Chemicals B.V., Deventer, Netherlands.

Semi-quantitative measurements were made of pH, protein, sugar, occult blood, ketones in pooled urine samples, using Labstix from Ames Laboratories and microscopy of sediment deposit - after centrifugation at 3000 rpm for 3 minutes. Deposits were examined for: erythrocytes, leucocytes, epithelial cells, amorph substances, phosphate crystals, casts, bacteria, worm eggs and sperm cells.

2.5.5. Pathology

All males still alive in week 135 and all females in week 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 a 4 % aqueous, neutral, phosphate-buffered formaldehyde solution: 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, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, skin, femur with joint and bone marrow, trachea, aorta, exorbital lachrymal glands, Zymbal's glands, cervix and uterus.

The organs which were 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 on 20 males and 20 females of the control group and of each of the two highest dose groups. For the control group these 20 males and 20 females comprised all animals killed at the end of the experimental period (14 males killed in week 135 and 19 females killed in week 144), supplemented by the animals which had lived the longest before they had to be killed in a moribund condition. For the two highest dose groups the rats subjected to detailed histopathology comprised the 20 males and the 20 females which had lived the longest before being killed in moribund condition.

Histopathological examination of all other rats, except for those killed after 26 and 52 weeks (see § 2.5.6. entitled: "Interim kills"), was restricted to the liver, the glands of Zymbal, the lungs, kidneys, spleen, pituitary, thyroid, adrenals, grossly visible tumours and organs containing gross lesions suspected of being tumours.

In order to be able to study the ultrastructure of an hepatic angiosarcoma, multiple liver samples were collected for electron microscopy when, on the basis of the gross autopsy findings, the liver was expected to contain an angiosarcoma. The samples were fixed by immersion in a fixative the composition of which is described in detail in § 2.5.6. on page 12. After verification of the presence of an angiosarcoma by light microscopy of paraffin-embedded, haematoxylin-eosin stained material, tissue samples from one male rat of the 14.1 mg/kg group were post-fixed and embedded in plastic, and semi-thin sections were prepared according to the methods described on page 12. Thereafter, ultrathin sections were prepared and viewed with a Philips EM 201G electron microscope at 60 kV.

2.5.6. Interim kills

Ten males and ten females of the control group and of the two highest dose groups were killed by decapitation and subjected to a thorough autopsy both after 26 and 52 weeks. The following observations were made in these rats either during the three weeks preceding their death or at post-mortem:

- blood-clotting-time using Normotest reagents from Nyogaard and Co., Oslo, Norway;
- serum electrolytes Na, K, Ca, and Mg, according to the method of Paschen and Fuchs (1971), and Cl according to a coulometric method;
- SAP, SGOT, SGPT, TSP, albumin and serum protein pattern, according to methods mentioned in § 2.5.3 on page 9;
- lactic dehydrogenase in the blood serum (LDH), using the method of Wróblewski and La Duc (1955);
- serum α-fetoprotein, according to a radio-immuno-assay described by Bockestein-Tjahjafi and Kroes (1976);
- liver function, using the bromosulphophthalein (BSP)-extinction test and the barbiturate sleeping-time method.
  Bromosulphophthalein (BSP) was injected intravenously (25 mg/kg body weight) and after exactly ten minutes blood was collected from the orbital sinus. The BSP-concentration in the serum was determined colorimetrically by measuring the absorbance at 580 nm.
  Sodium pentobarbital was injected intraperitoneally (25-48 mg/k;

body weight) and the sleeping-time was recorded according to Balazs and Grice (1963).

- kidney function, by means of the phenol-red excretion test, which was carried out according to a modification of the procedure described by Sharrat and Frazer (1963). Each rat was given an intramuscular injection of 0.1 mg of phenol-red (in saline) per kg body weight. The total urine which was produced in 60 minutes was collected, and the concentration of phenol-red was estimated colorimetrically by measuring the absorbance at 558 nm;
- mixed function oxidase activities, aminopyrine demethylase (APDM) and aniline hydroxylase (AH), in liver-preparations (after 52 weeks only). Immediately after killing the animals by decapitation a sample of each liver was taken and an S-9 fraction (=supernatant of homogenate centrifuged for 20 minutes at 9000 x g) was prepared. The S-9 fractions were stored at -20°C for 5 weeks before the enzyme activities were determined. APDM-activity was determined according to the procedure of Gram et al (1968) using the method of Nash (1953) as described by Cochin and Axelrod (1959) for measuring the amount of formaldehyde produced. AH-activity was determined by Gilbert and Goldberg (1965). Protein was determined by the method of Lowry et al (1951);
- weights of the liver and kidneys;
- histopathology of the liver, kidneys and glands of Zymbal;
- electron microscopy of the liver

  Liver samples of 2 males and 2 females of the control group, the 14.1 and 300 mg/kg groups were collected after 26 and 52 weeks for electron microscopical examination. The livers were fixed by perfusion through the portal vein under Nembutal anaesthesia. The fixative consisted of 1.5 % glutaraldehyde, buffered with 0.067 M sodiumcacodylate (pH 7.4), supplemented by 1 % sucrose. The perfusion flow was 5 ml/100 g/rat/minute. The perfusion of the liver with the fixative was started after perfusion with 0.9 % NaCl solution. The liver was removed from the body and a few slices from the left lobe were immediately cut in 1 mm^3 blocks, which were stored in the fixative overnight at 4°C. The blocks were post-fixed in 1 % OsO_4, buffered with 0.067 M sodiumcacodylate (pH 7.4) at 4°C, for a period of 17 hours.

Dehydration in graded aceton/water mixtures was followed by embedding in Epon 812.

One-micron sections were stained with Paragon.

The preparation and examination of ultra-thin sections were restricted to 1 female control rat, killed after 52 weeks; one male and one female of the 300 mg/kg group, killed after 26 weeks; and one female and two males of the 14.1 mg/kg group and two females and one male of the 300 mg/kg group which were killed after 52 weeks. Ultra-thin sections were stained with uranyl acetate and lead citrate and viewed with a Philips EM 200 or EM 201 G electron microscope at 60 kV.

2.6. Statistical analyses

Statistical analyses were carried out using Student's t-test for the changes in body weights and organ-to-body weight ratios; whereas haematological and biochemical values were evaluated by means of the test of Wilcoxon. The chi-square test was used for evaluating changes in mortality and in incidences of histopathological alterations.

The values obtained from animals of the highest dose group (300 mg/kg group) were not subjected to statistical analyses due to the fact that no proper corresponding control group was included in the study for practical reasons.

2.7. 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 inhalatory contact of the extra control rats with VCM. The rats in this additional control group were initiated a couple of weeks later (7 February 1975) and were from another (later) batch than the rats used in the other groups. In addition, the animals of the additional control group were fed the PVC-diet (the Institute's rat stock diet containing 10 % PVC-powder without VCM) *ad libitum*; this in contrast to the limited feeding-period of four hours each day which was employed for the other (real) control group.

Body weights, food consumption and mortality were recorded, and the figures are given in the present report. All animals were autopsied and a wide range of organs were preserved in 10 % buffered formalin;

but no slides were prepared.

3. RESULTS

3.1. Symptomatology

The behaviour of the rats during the first year of the experimental period was unremarkable, except for some respiratory difficulties in a few animals of the 300 mg/kg group. Most of these rats appeared to have severe and often necrotizing tracheitis and bronchopneumonia, which were probably the results of faulty dosing. During the second year the general condition of the rats in this group gradually declined and the administration of VCM by gavage became more and more difficult. Many rats grew lethargic and filthy before they died or were killed in a moribund condition. Most of these rats appeared to have severe lesions, including tumours, of the liver and lungs. It was therefore decided to terminate the VCM-treatment of this group in week 84.

After 18 months the number of unthrifty rats in the 5.0 and 14.1 mg/kg groups gradually increased; more rapidly in the 14.1 than in the 5.0 mg/kg 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 nodules could be detected in many of these rats by abdominal palpation. Animals with a swollen abdomen were occasionally seen; most of them appeared to have multiple intra-abdominal tissue masses which were always detected upon abdominal palpation. In addition, animals with breathing difficulties were seen quite often in these groups. Their lungs were invariably found to contain multiple nodules, which, upon microscopy, appeared to be primary angiosarcomas or metastases of either hepatic angiosarcomas or hepatocellular carcinomas.

External tissue masses were fairly often found; mainly after a test period of 18 months. The greater majority of the masses appeared to be mammary gland tumours, but there were also other types of tumours originating from the skin, the subcutis or dermal adnexa.

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

3.2. Body weights

The mean body weights of the rats of the 1.7, 5.0 and 14.1 mg/kg groups were very similar to those of the corresponding controls, indicating that the incorporation of VCM-containing PVC-powder in the diet did not adversely affect body-weight-gain (tables 3 and 4). However, the average body weights of the rats of the additional control group were clearly higher than those of the other groups receiving diets containing PVC-powder. This difference is undoubtedly due to the fact that the extra control animals had constant access to their diet, whereas the other rats had access to their diet only for a period of four consecutive hours each day.

The rats of the 300 mg/kg group which received VCM in oil by gavage had much higher body weights than the animals fed PVC-containing diets for a limited period of time daily (tables 3 and 4). An obvious explanation for this difference can be found in the fact that the former rats had constant access to stock diet, whereas the latter animals were allowed to eat stock diet containing 10 % (indigestible) PVC-powder for only four hours each day.

On the other hand, rats of the 300 mg/kg group had lower body weights than those of the additional control group, which points to an unfavourable effect of prolonged administration of VCM in oil by stomach tube (tables 3 and 4).

3.3. Food consumption

Food intake of rats fed the different PVC-diets for a limited period of time each day was similar (table 5). Food efficiency in these groups was also very similar (table 5).

In comparison with the rats fed the PVC-diets for four hours each day, the food consumption figures of the animals of the 300 mg/kg group were relatively low during the first period of 3 (females) to 9 months (males), and relatively high at later stages of the experimental period (table 5).

Food intake of the additional control rats receiving PVC-diet ad libitum was most often higher than that of the animals of the other groups (table 5).

3.4. Mortality

Mortality was low in males and females of the control group during the first two years of the experimental period; it amounted to 10 per cent after 2 years (table 6). This figure is only valid for the "real" control group, receiving the PVC-diet for a limited number of hours each day, because after 2 years the mortality among rats of the additional control group (fed ad libitum) was not less than three times higher (about 30 per cent). In females of the latter group death-rate remained relatively high during the final nine months of the test period; whereas in males, the differences in cumulative mortality between the two control groups gradually decreased and finally disappeared (table 6). This earlier mortality in rats of the additional control group is very probably connected with the higher body weights, and the higher and undoubtedly more frequent intake of food in this group.

A mortality of about 40 % occurred in males and females of the 300 mg/kg group, already after an experimental period of 18 months (table 6). Thereafter, mortality in this group rapidly increased, and shortly after 2 years all animals of the 300 mg/kg group were dead. Most of these rats died from pulmonary or hepatic insufficiency as a result of 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. Females of these groups died earlier than males (table 6).

In the low dose group (1.7 mg VCM/kg body weight), mortality of males was fully comparable to that of male control rats, and death-rate of females was only slightly higher than that of female controls (table 6).

3.5. Haematology

Haemoglobin-concentration and packed cell volume were found to be relatively low in the 14.1 mg/kg group after 78 and 94 weeks (table 7). At earlier stages of the experiment both parameters were similarly affected in the 300 mg/kg group; especially in males.

A relatively high number of white blood cells accompanied by a decrease in the percentage of lymphocytes and an increase in the percentage of neutrophils was observed in males of the 5.0 and 14.1 mg/kg

groups after 94 weeks (table 7). The same effects occurred in males and females of the 300 mg/kg group after 26 and 78 weeks, and in males of this group after 52 weeks. In contrast to these findings, the total number of leucocytes in the 300 mg/kg group was low after 13 weeks.

Blood of males and females of the 14.1 mg/kg group appeared to clot slightly more rapidly than that of controls (table 7). Blood-clotting-time of rats of the 300 mg/kg group was also relatively short.

3.6. Blood biochemistry

SGOT-activity showed a slight decrease in males of the 5.0 and 14.1 mg/kg groups and in females of the 14.1 mg/kg group after 13 weeks only (table 8).

The activity of GPT in the blood serum was slightly decreased in males of the 14.1 mg/kg group after 13 weeks, and slightly increased in females of this group after 26 and 52 weeks (table 8).

SAP-activity was slightly decreased in males of the 14.1 mg/kg group after both 13 and 26 weeks (table 8). Such a decrease in SAP-activity was also found in males and females of this group which were killed after 52 weeks (table 9). On the other hand, in the females of this group which were killed after 26 weeks, SAP-activity was slightly higher than in controls (table 9). A strikingly low SAP-activity was found in females of the 300 mg/kg group after 26 weeks (table 8).

A relatively low SGOT-activity, and fairly high SGPT- and SAP-activities occurred in the 300 mg/kg group after 13 weeks. At this stage of the experiment, the total serum protein and albumin contents were also relatively high in this group (table 8).

The urea nitrogen level of the blood was decreased in males of the 14.1 mg/kg group after an experimental period of 106 weeks (table 8).

The α-fetoprotein level in the blood serum was increased in males and females killed after 52 weeks (table 9). Males of the 300 mg/kg group which were killed after 52 weeks also had a relatively high α-fetoprotein content of the blood serum.

Electrolyte-contents of the blood serum are presented in table 10. No differences were found between the groups which were of toxicological

significance.

3.7. Urinalyses

No changes in the composition of the urine were found in rats fed on VCM-containing PVC-diets (table 11).

In general, the pH of the urine was fairly low in rats of the 300 mg/kg group (table 11). The amount of crystals in the urinary sediment of these rats was also relatively low from week 52 onwards.

Slightly increased UGOT-values were found in females of the 5.0 and 14.1 mg/kg groups after 13 weeks, and in females of the latter group after 52 and 94 weeks (table 12). Fairly high UGOT-values were also found in females of the 300 mg/kg group after 13 weeks, and in males and females of this group after 52 weeks.

Specific gravity of the urine was statistically significantly decreased in females of the various test groups receiving PVC-diets. There was, however, no dose-response relationship; and the differences are very probably the result of an unusually high specific gravity of the urine of controls.

Occasionally, high uric acid contents were found in rats of the 300 mg/kg group (table 12).

The phenol-red excretion test did not produce evidence of oral VCM exposure adversely affecting the kidney function (table 12).

3.8. Liver function

The results of the BSP-retention test and of the sleeping-time-determinations are presented in table 13. There were no clear indications that the ingestion of VCM resulted in a diminished function of the liver after experimental periods of 26 and 52 weeks.

3.9. Mixed function oxidase activities in the liver

No signicant differences in activity of APDM or AH in the liver were found between test groups and controls (table 14). With respect to the specific activities determined, this indicates that no induction of mixed function oxidase activities occurred following oral exposure to VCM.

3.10. Liver and kidney weights

Liver-to-body weight ratios were slightly increased in males and females of the 14.1 mg/kg group both after 26 and 52 weeks (table 15). In the 300 mg/kg group, relative liver weight was always higher than in the other groups. These findings suggest that ingestion of VCM may lead to a slight but definite liver enlargement.

Relative kidney weights were slightly increased in females of the 14.1 mg/kg group after 52 weeks only (table 15).

3.11. Pathology

3.11.1. Gross examination

Many rats exposed to VCM had severe liver lesions. Pronounced swelling, discolouration and altered consistency of one or more lobes, often containing varying numbers of cysts, were common findings. Nodules and nodule-like processes in the liver, widely varying in size (up to 4 cm in diameter), appearance and consistency, were frequently observed. 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 mostly present 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 on 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.

The abdominal cavity of several rats found dead contained blood, most probably derived from a ruptured cyst-like structure in the liver.

The above mentioned liver changes were most pronounced and occurred earliest and most frequently in the two highest dose groups. Angiosarcomatous nodules were not seen at all in the low dose group, and occurred more frequently in males of the 5.0 and 14.1 mg/kg groups than in females of these groups. The incidence of this type of nodules was high at the 300 mg/kg level, but there was no obvious difference in this respect between the sexes. Solid nodules occurred more frequently and were more often multiple in females of the 1.7, 5.0 and 14.1 mg/kg groups than in males of these groups. Actually, in males of the lowest dose group, liver nodules were seen in only a few cases.

Alterations in the lungs which could be ascribed to VCM-treatment consisted of small haemorrhagic or greyish nodules, or slightly protruding areas. The nodules were 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 which contained free blood or haemorrhagic fluid, and numerous pale, firm nodules (diameter 2 to 15 mm) in the peritoneum were encountered slightly more frequently in each of the three lowest dose groups (maximum incidence 15 per cent) than in the control or 300 mg/kg group (maximum incidence 5 per cent). Upon microscopy the nodules appeared to be mesotheliomas. In a few cases nodules with a similar gross appearance, occurring in the mesentery, were found to be an advanced stage of periarteritis nodosa. However, in contrast to the mesotheliomas, the arteritic nodules nearly always were seen, upon incision, to contain blood.

Other frequently occurring gross alterations were: slight to marked chronic respiratory disease; testicular atrophy; distended uterine horns; focal alopecia; large kidneys with a granular surface; white spots on the exorbital lachrymal glands; splenomegaly; ovarian cysts; mammary gland tumours; and tumorous enlargement of adrenals, thyroid and pituitary gland. Lesions seen in a relatively small number of rats included: enlarged seminal vesicles; haemorrhagic fluid in the urinary bladder; gastric haemorrhages; enlarged lymph nodes; and pale, enlarged cardiac auricles. In addition, a wide variety of pathological changes, including tumours, were observed in just one or a few rats. There was no indication that the occurrence of any of the neoplastic and non-neoplastic changes mentioned in the last paragraph were related to VCM.

3.11.2. Microscopic examination

3.11.2.1. Rats killed after 26 or 52 weeks (Interim kills)

Liver changes that could be attributed to VCM were found in rats which were killed after treatment periods of 26 or 52 weeks.

The hepatic alterations comprised foci of cellular alterations, neoplastic nodules, hepatocellular carcinomas and cystic bile duct hyperplasia (table 16). The hepatocellular lesions were classified according to the system described by Squire and Levitt (1975).

Only a few small, clear cell foci were found in a limited number

of test animals after 26 weeks (table 16). After 52 weeks the number of rats with foci of cellular alterations as well as the number of foci per liver had clearly increased. In addition, one male and two females of the 14.1 mg/kg group had a neoplastic nodule, and in one male and one female of the same group a hepatocellular carcinoma was found (table 16). It is remarkable that neither neoplastic nodules nor carcinomas were observed in the 300 mg/kg group. In this group the incidence of foci of cellular alterations was also clearly lower than in the 14.1 mg/kg group.

Cystic bile duct proliferation was only found in four out of the ten females of the 14.1 mg/kg group. Alterations attributable to VCM were not observed in the kidneys or glands of Zymbal.

3.11.2.2. Rats found dead or killed in moribund condition or terminally

The type and incidence of histopathological changes found in the liver are given in table 17, which shows that lesions attributable to VCM included foci of cellular alterations, neoplastic nodules, hepatocellular carcinomas, angiosarcomas, proliferation of atypical sinusoidal cells, extensive necrosis, cysts, liver cell polymorphism, centrolobular degeneration and extra medullary haematopoiesis.

The incidence of foci of cellular alteration in each of the three test groups receiving VCM-containing PVC-powder (the 1.7, 5.0 and 14.1 mg/kg groups) was much higher than in the control group, and also, 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 PVC-powder the incidence of both neoplastic nodules and hepatocellular carcinomas was (a) positively related to the VCM dose, and (b) much higher in females than in males.

Angiosarcomas of the liver were found in males and females of the three highest dose groups, but did not occur at all in controls and low dose animals (table 17). In both the 5.0 and 14.1 mg/kg group the incidence of angiosarcomas was three times as high 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 (table 17).

The probabilities for observation of liver tumours (neoplastic nodules, hepatocellular carcinomas and angiosarcomas) at death of the animals fed the various PVC-diets (viz. the 0, 1.7, 5.0 and 14.1 mg/kg groups) are plotted in figs. 4 and 5. A clear dose-response pattern is demonstrated by the considerable lengthening of the "latent period" for tumours of the liver with decreasing doses of VCM.

In the 300 mg/kg group, which for reasons of incomparability with the other groups was not included in figs. 4 and 5, the average latent period for the detection of liver tumours (almost exclusively angiosarcomas) at death was found to be 84 weeks for males and 83 weeks for females. For males and females of the 14.1 mg/kg group these average latent periods appeared to be 104 and 88 weeks respectively. The differences in the latent period between the two groups are clearly indicative of an earlier appearance of liver tumours in the 300 than in the 14.1 mg/kg 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 (table 17).

Large areas of necrosis were found in the liver of quite a high number of rats of the three highest dose groups (table 17).

Cysts, which were very probably lined by proliferated bile duct epithelium, were seen in a relatively high 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 (table 17). The size and multiplicity of the cysts varied widely among the individual animals.

Liver cell polymorphism was seen much more frequently in males of each of the test groups than in controls (table 17). In females, a slight degree of polymorphism was also observed in quite a number of controls, but the polymorphism was more pronounced in many rats from test groups than in controls.

Centrolobular liver degeneration was a frequent finding in females, but not in males, of the 300 mg/kg group (table 17).

Small foci of haematopoiesis in the liver were encountered more often in males and females of the two highest dose groups than in those of the other groups (table 17).

The non-neoplastic histopathological changes observed in organs other than the liver are presented in table 18.

A wide variety of alterations were found. Nearly all of them are considered to be related to the normal ageing process. An exception might be the very strong haematopoietic activity found in the spleen of 6 out of 40 males and 10 out of 40 females of the two highest dose groups (table 18, SPLEEN, 1). Whether this strong activity was related to the oral VCM exposure is doubtful, because marked haematopoiesis in the spleen is a quite common finding in old rats.

Several alterations were found clearly less frequently in each of the two highest dose groups than in controls. These included i.a. bronchiectasis in the lungs of males (table 18, LUNGS, 3), an increased amount of brown pigment in the red pulp of the spleen of females (table 18, SPLEEN, 2), focal infiltration of mononuclear inflammatory cells in the kidneys of females (table 18, KIDNEYS, 3), dilated mucosal glands in the stomach of females (table 18, STOMACH, 1), and endometritis or pyometra (table 18, UTERUS, 1). The lower incidence of these age-connected changes in the test groups can undoubtedly be ascribed to the fact that the test animals examined were much younger when killed (in extremis) than were the controls at the time of autopsy.

The site and type of the tumours observed, and their incidence in the different groups are presented in table 19.

The different aspects of the occurrence of tumours in the liver have already been described in this chapter (see also table 17).

Angiosarcomas were frequently found in the lungs at the two highest dose levels, and also occurred in a few rats of the 3.0 mg/kg group (table 19). They were most often seen as multiple very small to 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 angiosarcomas were encountered outside the lungs.

In four rats of the two highest dose groups an angiosarcoma was found in the abdominal cavity outside the liver (table 19, ABDOMEN, 2). In each of these rats the liver did not show signs of angiosarcoma-formation. Therefore, these tumours are considered primary extrahepatic angiosarcomas.

Pulmonary metastases of hepatocellular carcinomas were not uncommon (table 19). A total of 5 tumours of the glands of Zymbal (ceruminous glands) were found: two carcinomas in males of the 5.0 mg/kg group, and two carcinomas and one adenoma in rats of the 300 mg/kg group (table 19). Abdominal mesotheliomas were observed in each of the groups, the control group included (table 19). 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 an abdominal mesothelioma at death was found to decrease with increasing dose levels for both males and females.

The histological appearance of the peritoneal mesotheliomas was quite variable, but roughly two types could be distinguished, viz. a fibrous type in which sarcomatous areas predominated, and an epithelial type which mainly consisted 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 found much less frequently in females of the 5.0, 14.1 and 300 mg/kg groups than in controls or low dose animals (table 19). 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 as compared

to that of controls or of low dose animals. Despite the relatively low survival-time of females of the 14.1 and 300 mg/kg groups the number of females in each of these groups which bore adenocarcinomas of the mammary glands was twice as high as that in the control group. This might indicate that VCM enhances the development of mammary gland carcinomas.

The incidence of several other common types of tumours, known to be associated with old age, were found to decrease with increasing dose levels. Examples are: 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 (table 19).

3.12. Electron microscopy of the liver

3.12.1. Rats killed after 26 and 52 weeks (Interim kills)

Upon light microscopy of semi-thin sections, foci of hepatocytes and also scattered hepatocytes, containing a finely "vacuolized" cytoplasm, were found in rats of the 14.1 and 300 mg/kg groups both after 26 and 52 weeks. Ultrastructurally, the "vacuoles" appeared to represent swollen mitochondria with a pale matrix and short cristae. The abnormal mitochondria closely resembled those found in rats following inhalation exposure to 5000 ppm VCM for periods of 4-52 weeks (Feron et al, 1977); therefore, no further details of their ultrastructure are given here.

After 26 weeks small foci of hepatocytes, each containing numerous highly swollen, irregularly-shaped mitochondria without cristae and a matrix widely varying in density, were encountered in both livers examined. An increased amount of tubular, smooth endoplasmic reticulum (SER) was invariably present among the swollen mitochondria. Scattered individual hepatocytes were occasionally seen to contain only a few extremely swollen mitochondria but were otherwise normal the other mitochondria included.

After 52 weeks 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.

The sinusoidal cells generally looked quite normal. An occasional finding was interruption of the endothelial lining of a sinusoid. In such an area the endothelial cell processess were seen to be replaced by clusters of thrombocytes.

3.12.2. Ultrastructure of an hepatic angiosarcoma

Dilated sinusoids lined by tumour cells was the basic pattern of the tumour in the transitional zone between the tumour mass and the adjacent liver parenchyma. The tumour cells seemed to be pressed against the hepatocytes, thus obscuring the space of Disse. The tumour cells were slender and electron lucent, and contained a big nucleus with a small peripheral rim of chromatin and a large nucleolus. The perinuclear cytoplasm protruded into the sinusoidal lumen. Bristle-coated micropinocytotic vesicles were observed in the plasma membranes and the cytosol. There were only few cytoplasmic organelles such as mitochondria with or without membranous inclusions, free ribosomes and dilated single membranes occupied by ribosomes. Small elongated cell processess or angular cell partitions were often found between tumour cells of which the body with nucleus was visible and adjacent hepatocytes. Both features were interpreted as oblique sections through the slender parts of other surrounding tumour cells. These were surrounded by dark stained proteinaceous material from the blood serum. The angular cell partitions contained small mitochondria, many vesicles and single membranes of RER. The elongated cell processess were occasionally surrounded by osmiophilic granules, which also occurred in the peripheral cytoplasm of the adjacent hepatocytes. These granules closely resembled the granules which may occur in autophagosomes and tubular vesicles of SER under other pathological conditions.

The adjacent hepatocytes had partly lost their microvilli, which were replaced by an irregular plasma membrane. These hepatocytes were often packed with mitochondria which had dark and light areas and which were surrounded by a single strand of RER; they contained an increased amount of vesicular SER.

Large cells with an elongated nucleus were occasionally found. These cells invariably adjoined hepatocytes and were further in direct contact with bundles of either collagen fibres or fibres with a low contrast and without any striation.

No increase in fat-storing cells (Ito cells) was observed. An occasional pit-cell was found, which was recognized by its highly characteristic granules, organelle-free hyaloplasm and nucleus with dense chromatin. There was no indication that this type of cell was involved in the tumour process.

4. DISCUSSION

Only slight and often inconsistent differences were found between controls and test animals in several of the haematological and biochemical parameters applied in the present study. These slight deviations were considered to be of little, if any, toxicological significance, with two possible exceptions viz. shortening of the blood-clotting-time and increased α-fetoprotein levels in the blood serum.

Failure of the blood to clot has been noticed in guinea pigs that died during exposure to an atmosphere containing 40 per cent VCM (Mastromatteo et al, 1960) and in two workers of a PVC-factory which died from acute VCM-poisoning (Danziger, 1960). However, rats exposed to 5 or 2 per cent VCM for 19 or 92 days respectively had normal blood clotting times (Lester et al, 1963). In contrast to these findings in the present study and also in a previous inhalation study with VCM in rats (5000 ppm VCM, 6 hours a day, 5 days a week; Feron et al, 1977) 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 as seen in VCM-exposed rats used in our studies.

Müller et al (1976) recently reported a disturbed thrombocytic function in 9 out of 17 patients suffering from "vinyl chloride disease". Thrombocytopenia, which has been reported to be one of the symptoms of VCM-intoxication in man (Jühe et al, 1973; Lange et al, 1974; Müller et al, 1976) was observed neither in the present oral experiment nor in the previous one-year inhalation study (Feron et al, 1977). In future studies, it seems desirable to pay special attention

to the possible effects of VCM on thrombocytes.

Slight, though statistically significant, increases in the α-fetoprotein content of the blood serum were found in males and females of the 14.1 mg/kg group, which were killed after 52 weeks. After 26 weeks α-fetoprotein 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 weeks (Feron et al, 1977). These findings may indicate the presence of fetoglobulin-producing neoplastic or preneoplastic cells in the liver of rats exposed to VCM for a prolonged period of time. However, since the increase in α-fetoprotein level was only slight, it is more likely that aspecific liver damage, such as necrosis or degeneration of hepatocytes, is responsible for this slight effect.

The association between VCM and angiosarcoma in man has been recognized some years ago (Creech and Johnson, 1974; Lloyd, 1975). Several investigators have produced angiosarcomas in experimental animals through exposure to VCM either by inhalation or oral administration (Feron et al, 1977; Holmberg et al, 1976; Keplinger et al, 1975; Lee et al, 1978; Maltoni, 1975; 1976). The results of the present experiment show that oral exposure of rats to VCM at levels of 5.0 mg/kg body weight/day and higher caused hepatic angiosarcomas, pulmonary angiosarcomas (most 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.

Although hepatocellular tumours have been reported to occur in rats after treatment with VCM (Maltoni, 1976; Williamson, 1976) and some evidence exists as to the occurrence of hepatocellular carcinomas in persons exposed to VCM (Berk et al, 1976; Popper et al, 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 per cent 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 almost exclusively angiosarcomas at the very high dose level, via both angiosarcomas and hepatocellular tumours at the intermediate dose levels, to exclusively liver cell tumours at the lowest level. On the other hand, not only the dose but also the way of administering VCM might have been of significance for the difference in tumour response between the 300 mg/kg group and the lower dose groups, because gastric intubation of VCM in oil (once a day, 5 days a week) implies that daily the body (the liver) has to deal with a large amount of VCM in a short period of time, whereas in the case of oral intake of VCM-containing PVC-powder the body (the liver) is continuously (24 hours a day, 7 days a week) exposed to "fresh" VCM, which is gradually and uninterruptedly released from the PVC-powder during its transport through the gastro-intestinal tract. One might also speculate that the unexpectedly high incidence of liver cell tumours in test groups receiving PVC-powder is due to an unusual sensitivity of the liver cells to VCM as a consequence of an altered metabolic state caused by the reduction of the daily period of food intake to four hours. This restricted period of food intake might 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 (Watanabe et al, 1976a, 1976b, 1976c).

The lesions of the hepatic parenchyma that could be attributed to VCM did not only include "foci of cellular alterations", neoplastic nodules and carcinomas, but also necrosis, centrolobular degeneration and mitochondrial damage. In addition, the slight increases in α-fetoprotein content of the blood and in relative liver weight might also be indications of VCM injuring the hepatic parenchymal cells. Since it is known that tissue injury followed by repair (hyperplasia) may stimulate tumour formation, the possibility that the occurrence of hepatocellular tumours following VCM exposure is not due to the genotoxic activity of VCM but to the chronic process of continuous damage and repair, cannot be excluded. To elucidate the mechanism of action biochemical studies on VCM administered at low dietary levels under

conditions as prevailed in the present experiment seem to be indicated.

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, 1977; Popper et al, 1977). The liver changes found in the three lowest dose groups (1.7, 5.0 and 14.1 mg/kg 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 a precursor stage of focal hypertrophy and hyperplasia of the parenchyma occurred in only a very limited number of rats of this group.

Pulmonary angiosarcoma and extrahepatic angiosarcoma in other organs have been found in rats exposed to VCM by several investigators (Lee et al, 1978; Maltoni, 1975). In the present oral study extra-hepatic angiosarcomas were also seen, although it should be stressed that most of the pulmonary angiosarcomas are regarded as metastases from hepatic angiosarcomas.

Only a few test animals were found to bear a tumour of the glands of Zymbal. Since spontaneous Zymbal's gland tumours are rare and tumours of this organ are known to be induced in rats by VCM, it seems justified to ascribe the few Zymbal gland neoplasms found in the present experiment to the VCM-treatment.

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 in controls, which was considered insufficient evidence of VCM being capable of inducing this type of neoplasm in rats, but at most may be regarded as a slight indication of VCM enhancing carcinoma-formation in this organ.

To our knowledge, so far, VCM-induced mesothelioma have not been reported. In the present study an increased number of rats with abdominal mesothelioma were found in several of the test groups receiving PVC-powder. This might indicate that the ingestion of VCM has a potentiating effect on the development of mesothelioma in rats.

From the ultrastructural studies of the liver it appeared that foci of parenchymal cells affected by VCM scattered throughout the organ. Swelling of mitochondria was the earliest change observed and mitochondrial alterations remained characteristic features of the VCM-damage seen at later stages. In addition to swelling of mitochondria, prolonged treatment with VCM (26 and 52 weeks) appeared to cause a decrease in RER, loss of ribosomes from the RER, while the most important observation was an increase in SER. These findings suggest an enhancing effect of VCM on the so called mixed function oxidase system (MFO-system) which has indeed been shown to be involved in the detoxification of VCM (Bolt et al, 1976; Reynolds et al, 1975). However, the determinations of the aminopyrine demethylase and anilinehydroxylase activities in the liver did not produce evidence of VCM inducing MFO-activities.

The ultrastructural appearance of angiosarcoma-cells lining dilated sinusoids (elongated cells with slender processess forming a continuous lining of the sinusoid, presence of micropinocytotic vesicles) is suggestive of the tumour cells being derived from sinusoidal endothelial cells. In addition, there was no evidence that fat-storing cells, fibroblasts, Kupffer cells or pit-cells were involved in the tumour process. In contrast to the observations of other investigators (Popper and Thomas, 1975; Popper et al, 1977), neither an increase in fat-storing cells nor conspicuous fibrosis was found to precede hyperplasia of atypical sinusoidal cells. Therefore, in rats a fibrotic precursor stage does not seem to exist.

The high incidence of liver cell tumours in females of the low-dose group (27/58) clearly demonstrates the absence of both a "no-toxic-effect level" and a "minimum-effect level". For extrapolating the animal data to humans either a "no-toxic-effect level" or a "minimum-effect level" is essential. Further long-term studies with lower dietary VCM levels are, therefore, desirable.

5. CONCLUSIONS

From the results of the present study the following conclusions are drawn:

- incorporation of VCM-containing PVC-powder into the diet of rats is an effective method for studying the long-term effects of oral administration of VCM;
- VCM is a carcinogen in rats when administered by the oral route;
- the tumour response of the liver of rats to oral intake of VCM seems to shift from almost exclusively angiosarcomas at very high levels to exclusively hepatocellular tumours at low levels;
- the ingestion of VCM may enhance in rats the development of intra-abdominal mesotheliomas and of adenocarcinomas of the mammary glands;
- alterations of hepatocytes precede angiosarcoma development in the liver of rats;
- the "no-toxic-effect level" was lower than 1.7 mg VCM/kg body weight/day under the present rigorous conditions of continuous exposure for 24 hours a day.

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We, the undersigned, hereby declare that this work was performed under our supervision, according to the procedure herein described, and that this report represents a true and accurate record of the results obtained.

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Table 1. Percentage composition of the rat stock diet

Ingredients	%
Fish meal	7.0
Yellow maize	29.7
Whole wheat	36.0
Soya bean oil meal	11.0
Grass meal	3.0
Meat scraps	4.0
Dried whey	2.0
Brewer's yeast	3.0
Steamed bone meal	0.4
Soya bean oil	3.0
Trace mineralized salt [1)]	0.5
Vitamin ADEK-preparation[2)]	0.3
Vitamin B mixture [3)]	0.1

1) Percentage composition: MnO, 2.00; $ZnCl_2$, 0.50; KJ, 0.012; Co-acetate. $4H_2O$, 0.04; $FeSO_4.7H_2O$, 2.50; $CuSO_4.5H_2O$, 0.80; NaCl, 94.15.

2) Per gram preparation: 2100IU vitamin A as retinyl acetate; 700IU vitamin D as cholecalciferol; 15mg vitamin E as dl-α-tocopherol; and 1mg vitamin K as menaquinone sodium bisulphite

3) In mg per gram mixture: thiamine dichloride, 2.5; riboflavin, 3.0; pyridoxol hydrochloride, 5.0; nicotinic acid, 12.5; calcium dl-pantothenate, 7.5; d(+) biotin, 0.015; vitamin B_{12}, 0.005.

Table 2. Design and actual dosage levels of VCM in rats fed diets containing PVC-powder

Dose groups	Design VCM levels		Actual dietary VCM levels at the start of the feeding-period[1] (ppm)	Theoretical oral intake of VCM[2] (mg/kg body wt/day)	Actual oral intake of VCM[3] (mg/kg body wt/day)	Actual oral exposure level of VCM[4] (mg/kg body wt/day)
	ppm in diet	mg/kg body wt/day				
Control	0	0	0	0	0	0
Low	20	1	46	2.3	1.8	1.7
Mid	60	3	139	7.0	5.6	5.0
High	200	10	424	21.2	17.0	14.1

1) Average dietary VCM-contents determined immediately after preparation of the diets.

2) Oral intake of VCM if no loss of VCM by evaporation from the diets would occur (see also §2.2.1.2).

3) 80% of the theoretical oral intake (see also §2.2.1.2).

4) Oral intake of VCM diminished by the faecal VCM which was found to be 8, 10 and 17 per cent of the actual oral VCM intake for the low, mid and high dose groups respectively (see also §2.2.1.2). The VCM excreted in the faeces was considered to be still enclosed in the PVC-granules, and thus had not been in contact with the body.

Table 3. Mean body weights of males

week no.	mg VCM/kg body weight					
	0	1.7	5.0	14.1	300[1]	0[2]
0	92	91	90	92	113	62
1	101	101	99	98	151	103
2	138	138	137	136	189	137
4	182	183	182	177	233	212
6	211	215	213	207	262	263
8	238	241	242	235	276	295
10	250	255	261	250	307	348
12	269	273	277	268	324	ND[3]
16	283	289	299*	292	350	380
20	314	314	322	315	377	406
24	327	329	337*	331	386	425
28	332	333	346**	337	394	432
32	346	347	354*	346	403	437*
36	346	346	358*	350	400	444
40	350	353	360**	348	400	457
44	361	362	379***	364	416	462
48	367	375	380*	367	427	471
52	374	378	384	372	433	469
56	374	381	389**	372	426	483
60	386	391	398*	377	441	495
64	393	399	407	392	447	503
68	396	405	418*	396	459	515
72	399	406	412	395	457	521
76	402	414	415	404	460	524
80	399	414	419	403	453	528
84	403	414	419*	401	444	521
88	400	409	413	415	440	512
92	391	399	398	382	451	520
96	394	412*	413	387	453	517
100	384	407*	401	387	461	516
104	380	399*	395	381	438	495
108	373	391	392	376	-[4]	492
112	377	384	380	363	-	488

cont. ...

Table 3 cont.

week no.	mg VCM/kg body weight 0	1.7	5.0	14.1	300[1]	0[2]
116	361	375	384**	363	-	474
120	348	360	363	350	-	454
124	346	346	349	-	-	443
128	355	353	355	-	-	414
132	347	322	350	-	-	379
134	343	ND[3]	ND[3]	-	-	363

1) The figures of this group were not evaluated statistically (see §2.6).

2) Additional control group (see §2.7); the figures of this group were not evaluated statistically.

3) ND = not determined

4) - = all animals dead

*P<0.05; **P<0.01; ***P<0.001; according to the Student t-test

Table 4. Mean body weights of females

week no.	mg VCM/kg body weight					
	0	1.7	5.0	14.1	300[1)]	0[2)]
0	85	87	85	86	96	57
1	86	87	87	91	116	89
2	107	109	109	113	132	112
4	127	128	128	126	149	145
6	137	138	137	137	164	168
8	151	151	151	151	179	184
10	157	157	159	159	183	ND[3)]
12	163	163	165	164	188	202
16	172	171	173	171	202	215
20	182	182	185	180	210	225
24	189	190	191	186	212	231
28	191	192	195	189	216	238
32	198	198	201	195	221	239
36	200	201	203	197	224	246
40	200	200	203*	200	229	252
44	203	202	205	203	236	256
48	203	207	207	204	243	262
52	208	209	209	205	242	262
56	210	209	210	205	240	272
60	212	214	214	209	254	285
64	218	220	220	216	262	290
68	222	222	222	218	270	302
72	225	223	222	219	271	309
76	227	227	228	222	270	312
80	224	225	230	226	273	313
84	232	230	233	224	277	318
88	236	236	236	230**	278	323
92	229	230	233	212	271	325
96	235	237	244	253	275	326
100	231	236	235	230	270	328
104	231	230	238	224	270	332
108	234	231	225*	-	-[4)]	332
112	230	226	223	-	-	333

cont.....

Table 4 cont.

week no.	mg VCM/kg body weight 0	1.7	5.0	14.1	300[1]	0[2]
116	234	233	220	-	-	326
120	232	233	221	-	-	317
124	227	229	221	-	-	303
128	228	226	-	-	-	294
132	227	223	-	-	-	280
136	240	241	-	-	-	263
140	212	228	-	-	-	252
143	234	216	-	-	-	ND[3]

1) The figures of this group were not evaluated statistically (see §2.6).

2) Additional control group (see §2.7); the figures of this group were not evaluated statistically

3) ND = not determined

4) - = all animals dead

*P<0.05; **P<0.01; according to the Student t-test

Table 5. Average food consumption and food efficiency

mg VCM/kg body wt.	Food intake in g/rat/day in week: 1+2	3+4	10+11	24+25	36+37	60+61	72+73	84+85	Food efficiency during wk 1-4 gain(g)	food(g)	gain/food
					Males						
0	13.1	14.1	17.3	16.3	18.6	17.2	17.8	17.2	88.5	380.8	0.23
1.7	12.9	14.7	17.5	16.7	18.5	16.8	16.7	16.5	90.0	386.4	0.23
5.0	12.8	14.7	17.2	15.9	18.0	17.8	17.3	17.5	91.8	385.0	0.24
14.1	12.7	14.5	17.0	16.8	16.7	16.1	17.1	17.3	85.0	380.8	0.22
300	-[2]	-	17.1	15.3	17.8	19.0	19.6	-	-	-	-
0[1]	13.4	17.7	17.4	16.7	18.7	19.2	18.5	16.8	-	-	-
					Females						
0	10.3	10.8	11.7	10.3	11.0	11.9	11.8	11.3	41.2	295.4	0.14
1.7	10.6	10.8	11.5	10.4	11.1	11.6	11.3	11.5	40.7	299.6	0.14
5.0	10.6	10.7	11.6	10.9	11.7	11.2	11.4	11.1	43.6	298.2	0.15
14.1	10.2	10.7	12.0	11.0	12.0	11.0	11.4	11.9	40.4	292.6	0.14
300	-	-	10.3	12.3	13.4	13.2	15.4	-	-	-	-
0[1]	12.0	13.7	12.7	12.9	14.3	14.5	12.8	13.4	-	-	-

1) Additional control group (see §2.7)

2) - = not determined

Table 6. Cumulative mortality[1)]

group no.	mg VCM/kg body wt.	Number of deaths at end of week: 12	36	52	80	92	105	120	128	134[2)]	143[3)]
					Males						
7808	0	0	0	0	0	2	6	18	40	46	
7809	1.7	0	0	1	1	3	6	13	37	40	
7810	5.0	0	0	0	2	7	12	30*	49	60**	
7811	14.1	0	1	2	8**	22***	40***	56***	60***	60**	
7812[4)]	300	0	6	6	23	47	53	60	60	60	
7844[5)]	0	0	0	0	1	3	19	28	46	46	
					Females						
7808	0	0	0	0	1	5	6	22	27	32	41
7809	1.7	0	0	1	2	4	13	26	32	34	55**
7810	5.0	0	1	2	7*	16**	31***	55***	60***	60***	60***
7811	14.1	0	0	1	7*	43***	60***	60***	60***	60***	60***
7812[4)]	300	0	3	7	24	47	58	60	60	60	60
7844[5)]	0	0	0	1	4	10	17	27	42	43	52

1) Initial number of rats: 60/sex/group

2) The males still alive were killed in week 135

3) The females still alive were killed in week 144

4) The figures of this group were not evaluated statistically (see §2.6).

5) Additional control group (see §2.7); figures of this group were not evaluated statistically.

*P<0.05; **P<0.01; *** P<0.001; according to the Chi-square test

Table 7. Mean haematological findings in 10/rats/sex/group after 13, 26, 52, 78 and 94 weeks

mg VCM/kg body wt.	Haemoglobin (g/100ml)	Packed cell vol. (%)	Erythrocytes (10^6/mm^3)	Thrombocytes (10^3/mm^3)	Prothrombin time (sec) [1]	Leucocytes				
						Total (10^3/mm^3)	Differential count (%)			
							Lymph	Neutr	Eos	Mono
				Males: week 13						
0	15.6	47.6	8.2	766	-[2]	14.6	88.8	9.9	1.3	0
1.7	15.6	47.6	7.8	738	-	13.5	88.7	10.4	0.9	0
5.0	15.8	47.8	8.0	781	-	15.0	90.7	8.4	0.9	0
14.1	15.5	47.3	8.3	770	-	15.5	89.6	9.2	1.2	0
300[3]	15.3	44.9	8.3	906	-	12.0	82.6	16.0	1.4	0
				Females: week 13						
0	15.7	48.0	8.2	663	-	13.3	92.0	6.9	1.1	0
1.7	15.8	48.4	8.0	588	-	13.3	90.6	8.4	1.0	0
5.0	16.0	49.3	8.2	544*	-	13.6	94.7	5.0	0.3	0
14.1	16.2	48.3	8.2	656	-	13.8	92.0	7.6	0.3	0.1
300[3]	15.1	45.6	7.9	703	-	10.0	90.0	9.5	0.5	0
				Males: week 26						
0	15.7	48.3	7.5	744	41.3	10.7	87.0	11.6	1.1	0
1.7	15.5	47.8	7.5	741	-	11.0	83.3	15.5	1.2	0
5.0	15.4	46.3	7.7	708	-	10.6	85.7	13.1	1.2	0
14.1	15.3	47.7	7.8	615	38.9*	10.4	88.5	10.0	1.5	0
300[3]	14.8	45.1	7.7	751	35.2	11.5	72.9	24.2	2.9	0

cont.....

Table 7 cont.

mg VCM/kg body wt.	Haemoglobin (g/100ml)	Packed cell vol. (%)	Erythrocytes (10^6/mm^3)	Thrombocytes (10^3/mm^3)	Prothrombin time (sec)[1]	Leucocytes				
						Total (10^3/mm^3)	Differential count (%)			
							Lymph	Neutr	Eos	Mono
				Females: week 26						
0	15.3	47.9	7.8	700	37.2	9.8	79.9	18.8	1.3	0
1.7	15.7	48.9	7.8	689	-	9.1	74.5	22.5	3.0	0
5.0	15.6	48.1	7.9	600	-	9.7	78.7	19.1	2.2	0
14.1	15.4	48.6	7.9	657	34.1***	9.9	73.3	24.2	2.5	0
300[3]	15.0	47.3	7.8	665	31.7	10.2	64.3	32.1	3.6	0
				Males: week 52						
0	15.5	47.5	7.9	616	41.6	13.8	85.8	12.6	1.5	0.1
1.7	15.6	46.2	7.9	633	-	14.7	78.9	20.2*	0.9	0
5.0	15.7	48.5	8.3	637	-	12.3	85.6	13.7	0.7	0
14.1	15.2	46.9	8.2	724	38.8*	12.3	86.0	12.7	1.2	0.1
300[3]	14.8	45.4	8.2	600	37.3	15.3	77.9	21.0	1.1	0
				Females: week 52						
0	14.2	44.5	7.0	671	32.8	11.8	84.2	13.9	1.7	0.2
1.7	14.1	44.6	6.9	626	-	11.6	84.5	12.9	2.5	0.1
5.0	14.7	46.4	7.0	610	-	13.6	84.1	15.0	0.8	0.1
14.1	14.5	45.2	7.2	676	30.7	11.4	80.2	17.8	2.0	0
300[3]	14.6	45.3	7.0	756	30.5	12.5	81.5	17.2	1.3	0

cont.....

Table 7 cont.

mg VCM/kg body wt.	Haemoglobin (g/100ml)	Packed cell vol. (%)	Erythrocytes (10^6/mm^3)	Thrombocytes (10^3/mm^3)	Prothrombin time (sec) [1]	Leucocytes				
						Total (10^3/mm^3)	Differential count (%)			
							Lymph	Neutr	Eos	Mono
Males: week 78										
0	15.3	47.4	7.3	645	-	10.3	80.8	16.8	2.2	0.2
1.7	14.7	46.9	7.0	684	-	11.5	80.5	18.1	1.1	0.3
5.0	15.1	47.1	7.6	708	-	11.9	74.9	23.0	1.7	0.4
14.1	14.4*	45.4	7.3	779*	-	10.8	79.9	18.2	1.7	0.2
300	14.4	44.5	7.5	783	-	15.3	69.8	26.3	3.1	0.8
Females: week 78										
0	14.6	45.6	7.1	689	-	10.9	76.9	22.0	0.8	0.3
1.7	14.7	46.8	7.0	701	-	10.8	76.4	21.8	1.4	0.4
5.0	14.6	46.2	6.9	737	-	12.1	73.8	24.5	1.4	0.3
14.1	13.9	44.6	7.0	659	-	11.2	76.6	22.6	0.8	0
300 [3]	14.6	45.8	7.2	721	-	15.2	60.5	37.1	2.2	0.2
Males: week 94										
0	16.1	52.9	7.8	645	-	8.9	80.7	17.3	1.7	0.3
1.7	16.5	50.1	7.8	743	-	8.1	73.5	23.8	2.1	0.6
5.0	16.2	49.0	7.9	727	-	9.6	67.9*	29.0*	2.7	0.4
14.1	15.1**	45.8*	7.8	730	-	11.0*	68.4*	28.6*	2.3	0.7
300 [3]	-	-	-	-	-	-	-	-	-	-

cont.....

Table 7 cont.

mg VCM/kg body wt.	Haemo-globin (g/100ml)	Packed cell vol. (%)	Erythro-cytes (10^6/mm^3)	Thrombo-cytes (10^3/mm^3)	Prothrom-bin time (sec)[1]	Leucocytes				
						Total (10^3/mm^3)	Differential count (%)			
							Lymph	Neutr	Eos	Mono
Females: week 94										
0	14.9	46.0	7.4	630	-	11.2	67.7	30.6	1.6	0.1
1.7	14.7	45.8	7.0*	686	-	10.5	67.1	30.5	2.2	0.2
5.0	14.5	45.6	7.1	724	-	12.2	63.8	34.4	1.7	0.1
14.1	13.6	42.9	7.2	652	-	12.1	64.7	33.9	1.2	0.2
300[3]	-	-	-	-	-	-	-	-	-	-

1) Only determined in rats killed after 26 and 52 weeks (interim kills).

2) - = not determined

3) The figures of this group were not evaluated statistically (see §2.6).

*P<0.05; **P<0.01; ***P<0.001; according to the Wilcoxon test

Table 8. Mean biochemical blood values determined in 10 rats/sex/group after 13, 26, 52, 78 and 106 weeks

mg VCM/kg body weight	sugar (mg %)	BUN (mg %)	serum enzyme activities			serum proteins				
			GOT (RF-U)	GPT (RF-U)	AP (BL-U)	TSP (g %)	Albumin (g %)	Globulins (%)		
								α	β	γ
				Males: week 13						
0	84	18	130	27	8.1	7.0	2.6	55	32	13
1.7	84	17	136	32	9.3	6.8	2.6	52*	32	16
5.0	83	16	114*	26	7.7	6.8	2.6	55	31	14
14.1	84	16	104*	23**	6.6*	6.9	2.6	56	32	13
300[1)]	67	14	93	38	15.5	7.9	2.9	50	36	15
				Females: week 13						
0	77	13	89	21	7.2	6.8	2.8	45	35	19
1.7	74	14	92	20	6.3	7.0	2.9	45	35	20
5.0	78	12	89	19	7.7	6.7	2.8	42	34	24
14.1	79	13	77*	21	8.2	6.8	2.7	44	34	22
300[1)]	74	16	84	32	12.1	7.4	3.3	43	35	22
				Males: week 26						
0	77	14	79	26	11.0	7.3	2.7	55	29	16
1.7	75	13	84	27	9.6	7.3	2.7	57	27	16
5.0	79	14	69	26	10.0	7.4	2.7	57	28	15
14.1	81	14	83	26	8.7*	7.5	2.8	55	29	16
300[1)]	86	15	84	27	8.6	7.6	2.8	53	27	18

cont.....

Table 8 cont.

mg VCH/kg body weight	sugar (mg %)	BUN (mg %)	serum enzyme activities GOT (RF-U)	GPT (RF-U)	AP (BL-U)	serum proteins TSP (g %)	Albumin (g %)	Globulins (%) α	β	γ
				Females: week 26						
0	78	12	93	20	7.4	8.7	3.4	45	30	25
1.7	78	12	98	22	6.8	9.0	3.5	42	30	28
5.0	74	13	106	28	6.9	8.6	3.4	43	28	29*
14.1	78	13	106	25**	8.3	9.0	3.4	45	29	26
300[1)]	85	12	110	21	4.0	8.7	3.4	42	28	30
				Males: week 52						
0	87	11	80	24	7.2	6.2	3.2	49	28	24
1.7	85	11	79	23	8.1	6.3	3.0	48	29	23
5.0	88	11	87	26	8.8	6.5	3.1	48	30	22
14.1	88	11	90	23	6.4	6.7	3.2	48	29	23
300[1)]	72	12	86	23	9.1	7.0	3.2	48	28	24
				Females: week 52						
0	90	12	78	18	5.4	6.8	3.6	44	28	28
1.7	91	13	89	20	4.5	6.8	3.7	43	27	30
5.0	86	13	90	20	4.4	7.1	3.7	41*	28	31
14.1	89	12	87	21*	5.1	7.0	3.8	45	29	26
300[1)]	85	10	93	18	5.6	7.2	4.0	43	27	30

cont.....

Table 8 cont.

mg VCM/kg body weight	sugar (mg %)	BUN (mg %)	serum enzyme activities GOT (RF-U)	GPT (RF-U)	AP (BL-U)	serum proteins TSP (g %)	Albumin (g %)	Globulins (%) α	β	γ
			Males: week 106							
0	86	17	58	42	8.8	7.0	3.4	50	30	20
1.7	85	15	54	44	7.1	7.1	3.4	49	28	23
5.0	86	15	51	37	6.7	7.0	3.6	52	28	20
14.1	82	13**	70	50	7.5	6.9	3.3	52	30	19
300[1]	-[2]	-	-	-	-	-	-	-	-	-
			Females: week 106							
0	81	19	70	46	7.3	7.5	3.7	43	28	29
1.7	83	19	66	43	6.6	7.4	3.8	43	29	28
5.0	80	17	90	57	10.1	7.4	3.9	43	31	26
14.1	78	18	-	-	-	-	-	-	-	-
300[1]	-	-	-	-	-	-	-	-	-	-

1) The figures of this group were not evaluated statistically (see §2.6).

2) - = not determined because all or nearly all the animals were dead.

BUN = Blood urea nitrogen

GOT = Glutamic-oxalacetic transaminase

GPT = Glutamic-pyruvic transaminase

AP = Alkaline phosphatase

TSP = Total serum protein

RF-U = Reitman Frankel units

BL-U = Bessey Lowry Units

*P<0.05; **P<0.01; ***P<0.001; according to the Wilcoxon test

Table 9. Mean protein contents and enzyme activities in the blood serum of 10 rats/sex/group killed after 26 and 52 weeks (interim kills)

mg VCM/kg body wt.	serum proteins						serum enzyme activities			
	α-feto-protein[1] (ug/ml)	TSP (g %)	Albumin (g %)	Globulins (%) α	β	γ	GOT (RF-U)	GPT (RF-U)	AP (BL-U)	LDH (U/l)
Males: 26 weeks										
0	75	7.6	2.5	53	33	14	129	22	9.6	268
14.1	74	7.9	2.5	52	36*	13	119	20	8.2	272
300[2]	84	8.0	2.2	51	35	14	121	25	12.0	268
Females: 26 weeks										
0	99	7.8	2.7	46	32	22	159	20	8.2	270
14.1	91	7.5	2.4	50	28**	21	171	19	10.4*	311
300[2]	93	8.2	2.8	49	31	20	134	24	8.7	234
Males: 52 weeks										
0	30	5.8	2.5	48	32	20	79	23	10.4	163
14.1	51*	5.9	2.6	48	32	20	81	26	6.0***	166
300[2]	55	6.3	2.7	46	33	21	97	25	9.5	214
Females: 52 weeks										
0	53	6.0	3.0	43	30	26	89	22	7.0	149
14.1	109**	6.4	3.0	41	30	29	104	24	4.8*	100
300[2]	56	6.2	2.8	41	30	29	90	32	8.6	117

1) Number of animals examined varied from 4 to 8/sex/group.

2) The figures of this group were not evaluated statistically (see §2.6).

TSP = Total serum protein

GOT = Glutamic-oxalacetic transaminase

GPT = Glutamic-pyrovic transaminase

AP = Alkaline phosphatase

RF-U = Reitman-Frankel units

BL-U = Bessey-Lowry units

*P≤0.05; **P≤0.01; ***P≤0.001; according to the Wilcoxon test

Table 10. Mean electrolyte contents of the blood serum of 10 rats/sex/group killed after 26 and 52 weeks (interim kills)

mg VCH/kg body wt.	Males					Females				
	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cl (mg %)	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cl (mg %)
				Week 26						
0	3390	255	104	22	383	3611	256	101	22	371
14.1	3426**	268	102	22	376	3566	273*	104*	23	374
300[1)]	3429	270	105	23	349	3562	245	107	23	356
				Week 52						
0	3581	212	107	21	369	3556	202	105	22	361
14.1	3579	201	107	20	355	3471***	188	103*	21	371
300[1)]	3591	217	105	20	354	3474	181	103	21	363

1) The figures of this group were not evaluated statistically (see §2.6).

*P<0.05; **P<0.01; ***P<0.001; according to the Wilcoxon test

Table 11. Results of urine analyses carried out in pooled urine samples of 10 rats/sex/group after 13, 26, 52, 78 and 94 w

mg VCM/kg body wt.	appearance	pH	sugar	protein	occult blood	ketones	microscopic findings: RBC	WBC	epith	amorph	cryst	casts	bact	WE	sperm
Males: week 13															
0	yellow	6-7	–	+++	++	–	++	–	+	+	++	–	+	–	+
1.7	"	7-8	–	+++	–	–	+	–	+	+	+	–	+	–	+
5.0	"	7	–	++	++	–	++	–	±	++	++	–	+	–	+
14.1	"	7	–	+++	–	–	–	–	++	+	++	–	+	–	+
300	"	6	–	++	–	–	–	–	+	+	++	–	+	–	+
Females: week 13															
0	yellow	6	–	+	++	–	+	–	+	+	++	–	+	–	–
1.7	"	6-7	–	+	+	–	–	–	+	+	+++	–	+	–	–
5.0	"	6-7	–	+	–	–	–	–	+	++	++	–	+	–	–
14.1	"	7-8	–	+	–	–	–	–	+	+	++	–	+	–	–
300	"	6-7	–	+	–	–	–	–	+	+	+	–	+	–	–
Males: week 26															
0	yellow	7	–	+	–	–	–	–	±	+	++	–	+	–	+
1.7	"	7-8	–	+	–	–	–	–	+	+	+++	–	+	–	+
5.0	"	7	–	+	–	–	–	–	+	+	++	–	+	–	+
14.1	"	7-8	–	+	–	–	–	–	±	++	+++	–	+	–	+
300	"	6-7	–	++	–	–	–	–	+	+	+++	–	+	–	+
Females: week 26															
0	yellow	7	–	++	–	–	–	–	±	+	+++	–	+	–	–
1.7	"	7-8	–	++	–	–	–	–	+	+	+++	–	+	–	–
5.0	"	7	–	++	–	–	–	–	+	±	+++	–	+	–	–
14.1	"	7-8	–	++	–	–	–	–	±	+	+++	–	+	–	–
300	"	6	–	++	–	–	–	–	±	+	+++	–	++	–	–

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Table 11 cont.

mg VCH/kg body wt.	appearance	pH	sugar	protein	occult blood	ketones	microscopic findings: RBC	WBC	epith	amorph	cryst	casts	bact	WE	sperm
					Males: week 94										
0	yellow	7	–	++	–	–	–	–	+	++	+++	–	+	–	+
1.7	"	7-8	–	+++	–	–	–	–	±	++	+++	–	+	–	+
5.0	"	7	–	+++	–	–	–	–	+	++	+++	–	++	–	+
14.1	"	7	–	+++	–	–	–	–	+	++	++	–	+++	–	+
300	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
					Females: week 94										
0	yellow	7	–	+++	–	–	–	–	+	+	+++	–	+	–	–
1.7	"	7	–	+++	–	–	–	–	+	++	++	–	++	–	–
5.0	"	7	–	+++	–	–	–	–	+	+	++++	–	+	–	–
14.1	"	6-7	–	+++	–	–	–	–	±	+	++	–	+	–	–
300	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE

Grading system:
– = negative
± = minimal
+ = slight
++ = moderate
+++ = high
++++ = very high

RBC = red blood cells
WBC = white blood cells
WE = worm eggs
NE = not examined

Table II cont.

mg VCM/kg body wt.	appearance	pH	sugar	protein	occult blood	ketones	microscopic findings: RBC	WBC	epith	amorph	cryst	casts	bact	WE	sperm
Males: week 52															
0	Yellow	7	–	+++	–	–	–	–	+	±	++++	–	+	–	+
1.7	"	7	–	++++	+	–	+	–	+	±	++++	–	+	–	+
5.0	"	6-7	–	+++	–	–	–	–	+	±	++++	–	+	–	+
14.1	"	6-7	–	+++	–	–	–	–	+	±	++++	–	+	–	+
300	"	6	–	++++	–	–	–	–	+	+	+	–	+	–	+
Females: week 52															
0	Yellow	7	–	++	–	–	–	–	+	±	+++	–	+	–	–
1.7	"	7	–	++	–	–	–	–	+	±	+++	–	+	–	–
5.0	"	7-8	–	++	–	–	–	–	+	±	++++	–	+	–	–
14.1	"	7	–	++	±	–	–	–	+	±	+++	–	+	–	–
300	"	5-6	–	++	–	–	–	–	+	±	++	–	+	–	–
Males: week 78															
0	Yellow	7-8	–	++	–	–	–	–	+	+	+++	–	++	–	+
1.7	"	7	–	+++	±	–	–	–	+	+	+++	–	++	–	+
5.0	"	7-8	–	+++	–	–	–	–	+	++	++++	–	++	–	+
14.1	"	7	–	+++	–	–	–	–	+	++	++++	–	+++	–	+
300	"	6	–	+++	–	–	±	++	+	+	++	–	+	–	+
Females: week 78															
0	Yellow	8	–	+	–	–	–	–	±	+	+++	–	++	–	–
1.7	"	8	–	+	±	–	+	±	±	+	+++	–	++	–	–
5.0	"	8	–	+	–	–	–	–	±	+	++++	–	+	–	–
14.1	"	7	–	+	–	–	–	–	±	++	++	–	+++	–	–
300	"	6	–	+	–	–	±	++	+	+	+	–	++	–	–

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Table 12. Mean urinary findings in 10 rats/sex/group after 13, 26, 52, 78 and 94 weeks

mg VCM/kg body wt.	specific gravity	UGOT (R.F.-units)	volume (ml)	uric acid (µg/ml)	phenol red excretion in 1hr (%) 1)
		Males: week 13			
0	1.0685	15.9	2.7	-2)	-
1.7	1.0672	24.2**	2.4	-	-
5.0	1.0637	20.5	3.3	-	-
14.1	1.0681	21.1	3.0	-	-
300 3)	1.0659	12.7	2.3	-	-
		Females: week 13			
0	1.0805	14.9	1.6	-	-
1.7	1.0726*	19.6	1.8	-	-
5.0	1.0706**	23.5*	1.7	-	-
14.1	1.0727**	22.3**	1.9	-	-
300 3)	1.0757	25.7	1.4	-	-
		Males: week 26			
0	1.0474	25.0	3.3	753	29
1.7	1.0400	24.9	3.8	683	-
5.0	1.0485	21.2	3.4	740	-
14.1	1.0533	23.3	3.0	743	28
300 3)	1.0636	22.4	3.3	947	34
		Females: week 26			
0	1.0620	29.4	3.0	716	60
1.7	1.0582	26.5	2.9	641	-
5.0	1.0641	29.1	2.6	762	-
14.1	1.0639	27.6	3.2	635	57
300 3)	1.0619	28.2	2.3	857	68
		Males: week 52			
0	1.0643	18.8	4.1	833	41
1.7	1.0647	18.3	3.6	853	-
5.0	1.0655	16.3	3.8	839	-
14.1	1.0641	20.3	4.2	802	40
300 3)	1.0736	24.6	2.1	1305	36

cont....

Table 12 cont.

mg VCM/kg body wt.	specific gravity	UGOT (R.F.-units)	volume (ml)	uric acid (µg/ml)	phenol red excretion in 1hr (%) 1)
		Females: week 52			
0	1.0672	19.0	2.6	720	50
1.7	1.0626	18.0	2.9	676	-
5.0	1.0630	19.2	2.6	696	-
14.1	1.0647	23.3 *	3.1	689	57
300 3)	1.0694	26.0	2.7	899	62
		Males: week 78			
0	1.0563	26.1	3.9	776	-
1.7	1.0552	22.5	4.8	693	-
5.0	1.0547	24.0	5.0	690	-
14.1	1.0584	27.1	4.9	696	-
300 3)	1.0583	26.7	4.3	859	-
		Females: week 78			
0	1.0491	13.7	3.8	488	-
1.7	1.0471	14.6	4.1	479	-
5.0	1.0495	14.6	3.9	510	-
14.1	1.0500	14.9	3.9	621	-
300 3)	1.0361	11.3	5.8	498	-
		Males: week 94			
0	1.0607	10.4	4.8	746	-
1.7	1.0649	11.8	4.5	794	-
5.0	1.0632	10.0	5.3	780	-
14.1	1.0643	11.4	3.8	878	-
300 3)	-	-	-	-	-
		Females: week 94			
0	1.0615	9.2	2.6	614	-
1.7	1.0589	9.4	3.2	567	-
5.0	1.0574	11.0	3.2	577	-
14.1	1.0633	15.3	3.1	618	-
300 3)	-	-	-	-	-

1) Only determined in rats killed after 26 and 52 weeks (interim kills).

2) - = not determined

3) The figures of this group were not evaluated statistically (see 2.6).

UGOT = urine glutamic-oxaloacetic transaminase; R.F.-units = Reitman-Frankel units; * P<0.05; ** P<0.01; *** P<0.001; according to the Dixon test

Table 13. Results of liver-function tests carried out in 10 rats/sex/group killed after 26 and 52 weeks

mg VCM/kg body wt.	Males		Females	
	BSP-extinction after 10 min. ($x10^3$)	sleeping time (min)	BSP-extinction after 10 min. ($x10^3$)	sleeping time (min)
	Week 26			
0	284	126	194	94
14.1	195*	105	229	120
300[1]	210	108	113	81
	Week 52			
0	354	125	176	94
14.1	303	108	155	85
300[1]	337	103	247	103

1) The figures of this group were not evaluated statistically (see § 2.6)
*$P<0.05$; according to the Wilcoxon test.

Table 14. Average aminopyrine demethylase (APDM)- and anilinehydroxylase (AH) activities in S9 fractions of the livers of rats killed after 52 weeks (interim kill)

mg VCM/kg body wt.	Males		Females	
	Specific activity of:		Specific activity of:	
	APDM (µmol formaldehyde/30'/g protein	AH (µmol aminophenol/30'/g protein	APDM (µmol formaldehyde/30'/g protein	AH (µmol aminophenol/30'/g protein
0	36.1±5.1[1] (6)[2]	7.0±0.5 (5)	26.7±5.4 (5)	8.3±0.5 (5)
14.1	35.0±4.8 (6)	6.6±0.7 (6)	18.7±3.9 (7)	6.3±0.4 (5)
300	23.2±7.0 (4)	7.1±0.4 (7)	24.1±4.6 (5)	7.5±0.6 (4)

1) Standard error of the mean
2) The number of animals examined is given in brackets

Table 15. Average body weights and relative weights of liver and kidneys of 5 to 10 rats/sex/group killed after 26 and 52 weeks

mg VCM/kg body wt.	Males			Females		
	Body (g)	Liver (% of body wt)	Kidneys (% of body wt)	Body (g)	Liver (% of body wt)	Kidneys (% of body wt)
			Week 26			
0	329	2.54	0.62	194	2.60	0.66
14.1	323	2.91*	0.64	190	3.08**	0.67
300[1)]	379	3.31	0.65	212	3.86	0.74
			Week 52			
0	372	2.63	0.56	200	2.57	0.66
14.1	380	3.00	0.56	195	3.31*	0.69***
300[1)]	432	3.38	0.59	240	3.44	0.64

1) The figures of this group were not evaluated statistically (see 12.6)

*P<0.05; **P<0.01; ***P<0.001; according to the Student t-test

Table 16. Type and incidence of treatment-related hepatic changes found in rats killed after 26 weeks and 52 weeks (interim kills)

Type of lesions	Incidence of lesions					
	males			females		
	mg VCM/kg body wt[1]			mg VCM/kg body wt[1]		
	0	14.1	300	0	14.1	300
Animals killed after 26 weeks						
No. of animals examined	10	10	9	10	10	10
1. Clear cell foci (small):						
a. one or few	0	1	1	0	5**	2
Animals killed after 52 weeks						
No. of animals examined	9	10	9	9	10	8
1. Clear cell foci (small):						
a. one or a few	1	3	5	0	1	4
b. several	0	5**	0	0	8**	0
2. Basophilic foci (small):						
a. one or a few	0	0	0	0	4	1
3. Eosinophilic foci (small):						
a. one or a few	0	2	0	0	5**	0
4. Neoplastic nodule	0	1	0	0	2	0
5. Hepatocellular carcinoma	0	1	0	0	1	0
6. Cystic proliferation of bile duct epithelium	0	0	0	0	4*	0

1) The figures of this group were not evaluated statistically (see§2.6).
*$P<0.05$; **$P<0.01$; according to the Chi-square test.

Table 17. Type and incidence of histopathological changes in the liver [1]

Type of changes [2]	Incidence of changes									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300 [4]	0	1.7	5.0	14.1	300 [4]
Number of animals examined [3]	55	58	56	59	55	57	58	59	57	54
I. FOCI OF CELLULAR ALTERATION										
a. CLEAR CELL FOCI										
I. ONE OR A FEW	0	8**	16***	21***	9	4	24***	22***	36***	10
II. SEVERAL TO MANY	0	1	0	0	0	0	0	0	0	0
b. BASOPHILIC FOCI										
I. ONE OR A FEW	8	15	19*	22**	12	10	33***	17	28***	19
II. SEVERAL TO MANY	0	3	2	0	0	0	0	0	0	0
c. EOSINOPHILIC FOCI										
I. ONE OR A FEW	3	20***	24***	33***	10	8	35***	20*	29***	6
II. SEVERAL TO MANY	0	3	3	0	1	0	0	0	0	0
2. NEOPLASTIC NODULES										
I. ONE	0	1	7**	14***	3	2	17***	23***	8	1
II. FEW TO SEVERAL	0	0	0	9**	0	0	9**	16***	36***	1
3. HEPATOCELLULAR CARCINOMA	0	1	2	8**	1	0	4	19***	29***	0
4. ANGIOSARCOMA	0	0	6*	27***	27	0	0	2	9**	29
5. PROLIFERATION OF ATYPICAL SINUSOIDAL CELLS ONLY	2	0	4	7	6	4	6	3	4	7
6. EXTENSIVE AREAS OF NECROSIS	4	4	8	23***	21	5	6	19**	27***	24
7. CYSTS	2	3	4	16***	3	9	30***	41***	49***	3
8. LIVER-CELL POLYMORPHISM a. slight	4	14*	21***	34***	30	30	30	30	13**	38
b. moderate/marked	0	2	7**	8**	6	4	21***	8	28***	3

cont.....

Table 17 cont.

Type of changes [2]	Incidence of changes									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
9. CENTROLOBULAR LIVER-CELL DEGENERATION*	0	0	0	1	1	1	2	3	1	18
10. SLIGHT EXTRA-MEDULLARY HAEMATOPOIESIS	0	1	0	10**	8	1	3	1	6	12
11. Foci of RES-cells occasionally accompanied by a few necrotic hepatocytes	12	9	6	11	6	15	8	8	8	7
12. Periportal infiltrates of mononuclear cells	20	16	13	11*	20	19	15	14	14	12
13. Slight degree of bile duct proliferation	9	4	3	9	11	15	9	14	14	3
14. Periportal fibrosis	3	3	2	3	8	3	3	3	6	1
15. Single cell necrosis a. slight	10	7	7	15	16	6	14	4	3	2
b. moderate	3	4	1	4	0	1	6	3	1	2
16. Vacuolization of hepatocytes, mainly: a. focal	8	17	6	8	10	5	6	9	11	13
b. diffuse	1	0	2	0	1	4	3	0	2	0
17. Distended sinusoids	2	0	0	0	1	0	0	0	0	2
18. Perihepatitis	0	0	0	0	0	1	1	0	0	0
19. Large abscess	0	0	0	0	0	0	1	0	1	0
20. Kupffer cell sarcoma	0	0	1	0	0	0	0	0	0	0
21. Reticulum cell sarcoma	1	1	0	0	0	0	0	1	0	0
22. Fibrosarcoma	0	0	1	0	0	0	0	1	0	0
23. Haemangioendothelioma	0	0	0	0	1	0	0	0	0	0
24. Mesenchymal type of tumour	0	0	1	0	0	0	0	0	0	0

1) Treatment-related changes are written in capitals. 2) Specific hepatocellular lesions, viz. those mentioned in this table under numbers 1, 2 and 3, were classified according to Squire and Levitt (1975). 3) The initial number of animals was 60/sex/group. A number of rats could not be examined because of cannibalism or advanced autolysis. 4) The figures in this group were not evaluated statistically (see §2.6).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, according to the Chi-square test.

Table 18. Site, type and incidence of non-neoplastic histopathological changes observed in animals of the control and two highest dose groups[1)]

Site and type of changes	Incidence of changes					
	males			females		
	mg VCM/kg body wt			mg VCM/kg body wt		
	0	14.1	300[2)]	0	14.1	300[2)]
Number of animals examined	20	20	20	20	20	20
LUNGS						
1. Adenomatoid lesions	0	1	0	0	0	1
2. Peribronchial, periobronchiolar and/or perivascular lymphoid aggregates						
a. slight	7	9	10	11	16	13
b. moderate	8	5	2	5	2	0
c. marked	2	5	0	0	0	0
3. Chronic respiratory disease a. slight	9	2*	3	1	1	0
b. moderate/marked	2	0	1	2	0	0
4. Focal accumulations of alveolar macrophages	2	0	0	4	0	0
5. Focal proliferative pneumonitis	0	0	0	1	0	0
6. Focal, increased cellularity of interalveolar septa	0	1	0	0	0	0
SPLEEN						
1. Increased haematopoietic activity	0	4	2	0	4	6
2. Increased amount of brown pigment in the red pulp	0	0	0	9	3*	2
3. Depletion of the white pulp	1	1	1	0	0	0
4. Reticulum cell hyperplasia	0	0	1	0	1	0
5. Focal fibrosis	0	0	0	0	0	1
KIDNEYS						
1. Tubular nephrosis a. slight	7	8	4	12	10	8

cont.....

Table 18 cont.

Site and type of changes	Incidence of changes					
	males			females		
	mg VCM/kg body wt			mg VCM/kg body wt		
	0	14.1	300	0	14.1	300
Kidneys cont.						
1. Tubular nephrosis b. moderate	8	5	6	5	2	3
c. marked	4	4	8	1	0	2
2. Degenerated glomeruli	12	8	13	5	1	2
3. Focal infiltrates of mononuclear inflammatory cells	6	0**	5	9	1**	1
4. Periglomerular sclerosis	0	0	1	0	0	0
5. Increased amount of brown pigment within cortical tubular epithelial cells	2	2	0	5	4	3
6. Calcareous deposits in the cortico-medullary layer and/or papilla	6	2	1	6	1*	5
7. Dilatation of tubules in: a. cortex	4	4	9	8	1**	2
b. medulla	0	3	6	2	0	3
8. Cortical cyst lined by flattened epithelium	0	1	0	0	0	0
9. Pyelitis	0	0	1	0	0	0
10. Focal proliferation of atypical tubular epithelium cells	0	0	1	0	0	0
11. Hydronephrosis	0	0	0	0	1	0
PANCREAS						
1. Focal infiltrates of mononuclear inflammatory cells	1	0	0	0	0	2
2. Periarteritis	2	0	0	1	0	0
3. Foci of cystic ductuli	1	0	0	0	0	0
4. Change of acini into duct-like structures	0	0	1	0	1	0
HEART						
1. Focal infiltrates of mononuclear inflammatory cells	3	0	0	2	0	1
2. Focal myocarditis or myofibrosis	4	2	0	0	1	2
3. Slight pericardial fibrosis	0	0	1	0	0	0

cont.....

Table 18 cont.

Site and type of changes		Incidence of changes					
		males			females		
		mg VCM/kg body wt			mg VCM/kg body wt		
		0	14.1	300	0	14.1	300
NOSE							
1. Subepithelial infiltrates of mononuclear inflammatory cells		0	1	0	6	1*	1
2. Atypical metaplasia of neuroepithelial cells		0	0	1	0	0	0
3. Proliferation and degeneration of Bowman's glands		0	0	1	0	0	0
TRACHEA							
1. Subepithelial infiltrates of mononuclear inflammatory cells	a. slight	6	6	6	8	6	6
	b. moderate	7	4	2	8	0**	0
OESOPHAGUS							
1. Peri-oesophagitis		1	0	0	0	0	0
STOMACH							
1. Dilated mucosal glands		8	6	7	14	3***	7
2. Hyperkeratosis in the fore stomach		2	0	0	0	0	2
3. Submucosal infiltrates of polymorphonuclear inflammatory cells		0	0	1	0	0	1
ADRENALS							
1. Degenerative cortical changes (haemorrhagic cysts and parenchymal necrosis)		5	0*	0	10	6	7
2. Increased vacuolization of cortical cells:	a. diffuse	2	3	4	2	0	0
	b. focal	11	5	3	5	6	3
3. Foci of basophilic medullary cells		1	2	2	0	0	0
4. Slight mononuclear cell infiltrates in cortex and/or medulla		1	0	0	0	1	2
5. Lipofuscine-like pigment in the cortical cells		0	0	0	4	1	0
PITUITARY							
1. Cyst:	a. in pars distalis	3	1	1	1	0	1
	b. in pars intermedia	1	0	0	0	1	0
2. Hyperplasia of chromophobe cells		1	0	1	1	0	0

cont....

SL 042013

Table 18 cont.

Site and type of changes		Incidence of changes					
		males			females		
		mg VCM/kg body wt			mg VCM/kg body wt		
		0	14.1	300	0	14.1	300
BRAIN							
1. Focal submeningial infiltrates of mononuclear inflammatory cells		1	0	0	0	0	0
SCIATIC NERVE							
1. Demyelinization		1	0	0	0	0	0
THYROID							
1. Activated appearance		7	7	8	1	6*	3
2. Proliferation of parafollicular cells	a. diffuse	0	0	1	0	0	0
	b. focal	0	0	1	2	0	0
3. Psammoma bodies		1	0	0	0	0	0
SKELETAL MUSCLE							
1. Focal infiltrates of mononuclear inflammatory cells		2	0	0	0	0	0
2. Focal myodegeneration		3	0	0	0	0	0
3. Haemorrhages		1	0	1	0	0	0
EXTRAORBITAL LACHRYMAL GLANDS							
1. Prosoplasia of glandular epithelium into Harderian-type acini		16	17	16	0	0	0
2. Focal infiltrates of mononuclear inflammatory cells		7	2	1	0	0	0
HARDERIAN GLANDS							
1. Adenitis		6	1*	1	3	0	1
2. Calcareous deposits		0	2	0	0	0	0
PAROTID SALIVARY GLANDS							
1. Vacuolization		0	0	0	1	0	0
2. Desquamation of ductular epithelium		0	0	1	0	0	0

cont.....

Table 18 cont.

Site and type of changes	Incidence of changes					
	males			females		
	mg VCM/kg body wt			mg VCM/kg body wt		
	0	14.1	300	0	14.1	300
SUBMAXILLARY SALIVARY GLANDS						
1. Change of acini into duct-like structures	1	0	0	0	0	0
2. Desquamation of ductular epithelium	0	0	1	0	0	0
SUBLINGUAL SALIVARY GLANDS						
1. Desquamation of ductular epithelium	0	0	1	0	0	0
ZYMBAL'S GLANDS						
1. Cystically extended ducts	1	0	0	0	0	0
INTESTINES						
1. Parasites	3	1	1	1	0	2
URINARY BLADDER						
1. Parasites	0	0	1	0	0	0
2. Proteinaceous plug	6	2	2	0	0	0
3. Hyperplastic epithelium	1	0	0	0	0	0
MESENTERY						
1. Periarteritis	2	0	0	0	0	0
2. Peritonitis	0	2	0	0	0	0
OVARIES						
1. Follicular cysts				5	0*	5
TESTES						
1. Atrophy: a. a few to several atrophic tubules	5	2	1			
b. unilateral atrophy	0	3	4			
c. bilateral atrophy	5	3	1			
2. Infiltrates of mononuclear inflammatory cells	1	0	0			

cont....

Table 18 cont.

Site and type of changes	Incidence of changes					
	males			females		
	mg VCM/kg body wt			mg VCM/kg body wt		
	0	14.1	300	0	14.1	300
Testes cont.						
3. Periarteritis	2	0	0			
EPIDIDYMIDES						
1. Focal infiltrates of mononuclear inflammatory cells	0	1	2			
2. Periarteritis	1	0	0			
3. Epididymitis	1	0	0			
4. Intraepithelial cysts	1	0	0			
PROSTATE						
1. Prostatitis	5	0*	0			
2. Postatrophic hyperplasia	0	1	0			
UTERUS						
1. Endometritis/pyometra				11	2**	1
2. Dilated uterine horn				0	2	1
3. Cystic endometrial hyperplasia				4	1	1
4. Polyp				2	2	1
MAMMARY GLANDS						
1. Focal infiltrates of mononuclear inflammatory cells				1	0	0
2. Duct ectasia				2	0	1
3. Abscess				1	0	0

1) The changes observed in the liver are not included in this table; they are given in table 17.
2) The figures in this group were not evaluated statistically (see §2.6).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, according to the Chi-square test.

SL 042016

Table 19. Site, type and incidence of tumours in the different groups of rats [1]

Site and type of tumours	Incidence of tumours									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300 [3]	0	1.7	5.0	14.1	300
Initial number of animals	60	60	60	60	60	60	60	60	60	60
Effective number of animals	55	58	56	59	55	57	58	59	57	54
Number of animals bearing primary tumours	38	50	49	52	44	54	56	55	57	47
Total number of primary tumours	67	97	92	121	85	101	140	129	134	93
LIVER [2]										
1. NEOPLASTIC NODULES a. ONE	0	1	7**	14***	3	2	17***	23***	8	1
b. A FEW TO SEVERAL	0	0	0	9**	0	0	9**	16***	36***	1
2. HEPATOCELLULAR CARCINOMA	0	1	2	8**	1	0	4	19***	29***	0
3. ANGIOSARCOMA	0	0	6*	27***	27	0	0	2	9**	29
4. Kupffer cell sarcoma	0	0	1	0	0	0	0	0	0	0
5. Reticulum cell sarcoma	1	1	0	0	0	0	0	1	0	0
6. Fibrosarcoma	0	0	1	0	0	0	0	1	0	0
7. Haemangioendothelioma	0	0	0	0	1	0	0	0	0	0
8. Mesenchymal type of tumour	0	0	1	0	0	0	0	0	0	0
LUNGS										
1. ANGIOSARCOMA	0	0	4*	19***	19	0	0	1	5*	23
2. METASTASIS OF HEPATOCELLULAR CARCINOMA	0	0	0	1	0	0	0	10**	4	0
3. Adenoma	0	0	0	0	1	0	0	0	0	0
4. Metastases of squamous cell carcinoma skin	0	1	0	0	0	0	0	0	0	0
5. Metastases of fibrosarcoma	0	0	0	0	0	0	0	1	0	0
6. Metastases of reticulum cell sarcoma	0	0	0	0	0	0	0	1	0	0

cont.....

Table 19 cont.

Site and type of tumours	Incidence of tumours									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
LUNGS cont.										
7. Metastases of mesenchymal liver tumour	0	0	1	0	0	0	0	0	0	0
8. Metastases of adenocarcinoma mammae	0	0	0	0	0	0	0	0	1	0
9. Metastases of Kupffer cell sarcoma	0	0	1	0	0	0	0	0	0	0
10. Metastases of mesothelioma	0	0	0	0	0	0	1	0	0	0
ZYMBAL'S GLANDS										
1. SQUAMOUS CELL CARCINOMA	0	0	2	0	1	0	0	0	0	1
2. Adenoma	0	0	0	0	0	0	0	0	0	1
ABDOMEN										
1. MESOTHELIOMA	3	1	7	8	1	1	6	3	3	0
2. ANGIOSARCOMA	0	0	0	0	1	0	0	0	2	1
3. METASTASES OF HEPATOCELLULAR CARCINOMA	0	0	0	0	0	0	0	1	0	0
4. Fibrosarcoma	0	0	0	3	0	1	2	0	0	0
5. Osteosarcoma	0	0	0	0	1	0	0	0	1	0
6. Sarcoma	0	0	3	1	0	0	0	1	0	0
7. Reticulum cell sarcoma	0	1	0	0	1	0	0	0	0	0
8. "Schwann cell tumour"	0	0	0	0	0	0	1	0	0	0
9. Unclassified abdominal tumour	0	0	0	0	0	2	0	0	0	0
SPLEEN										
1. Haemangioendotheliosarcoma	0	1	0	0	0	0	0	0	0	0
2. Lymphosarcoma	0	0	0	0	0	0	0	0	0	1
NOSE										
1. Squamous cell carcinoma	0	0	0	1	0	0	0	0	0	0

cont.....

Table 19 cont.

Site and type of tumours	Incidence of tumours									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
BRAIN										
1. Granular cell myoblastoma	1	1	0	0	0	0	1	0	0	0
2. Oligodendroglioma	1	0	0	0	0	0	0	0	0	0
3. Plexus papilloma	0	0	0	0	0	0	1	0	0	0
4. Glial cell tumour	0	0	2	0	1	0	0	0	0	0
5. Ependymoma	0	0	0	1	0	0	0	0	0	0
6. Mesodermal tumour	0	0	1	0	0	0	0	0	0	0
PANCREAS										
1. Adenocarcinoma	0	0	0	0	0	1	2	1	0	0
THORAX										
1. Mesothelioma	1	0	0	0	0	0	0	0	0	0
THYROID										
1. Parafollicular cell adenoma	4	12*	10	3	3	7	10	3	2	0
2. Parafollicular cell carcinoma	1	0	1	0	0	0	0	0	0	0
3. Follicular cell adenoma	0	0	0	1	0	1	0	0	1	0
ADRENALS										
1. METASTASES OF HEPATOCELLULAR CARCINOMA	0	0	0	0	0	0	0	1	0	0
2. Cortical adenoma a. small	15	17	10	6*	7	8	11	14	14	9
b. medium-sized	3	6	6	4	2	7	9	2	1	2
c. large	0	2	1	0	0	11	10	4*	2*	3
3. Benign phaeochromocytoma a. small	6	11	6	3	2	2	1	1	0	0
b. medium-sized	4	6	0	1	4	0	0	1	0	1

cont.....

Table 19 cont.

Site and type of tumour		Incidence of tumours									
		males					females				
		mg VCM/kg body wt					mg VCM/kg body wt				
		0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
Adrenals cont.											
3. Benign phaeochromocytoma	c. large	0	1	1	0	0	0	0	0	0	1
4. Malignant phaeochromocytoma		1	3	1	0	0	0	0	0	0	0
PITUITARY											
1. Chromophobe adenoma		2	12**	3	0	0	12	14	5	3*	2
2. Acidophilic adenoma		7	5	1*	2	0	0	1	0	1	0
3. Cystic adenoma		3	8	2	0	0	2	1	5	1	1
4. Chromophobe carcinoma		1	0	1	0	0	3	0	2	0	0
BLOOD											
1. Monocytic leukemia		0	0	0	0	1	1	1	1	0	0
2. Myeloid leukemia		0	0	0	0	0	0	1	0	0	0
3. Lymphocytic leukemia		0	0	1	0	1	0	0	0	0	1
4. Unclassified leukemia		1	0	0	1	1	0	0	0	0	0
HEART											
1. "Endocardial Disease" [4]		2	0	2	2	1	1	0	0	0	1
2. Haemangioendotheliosarcoma		1	0	0	0	0	0	0	0	0	0
KIDNEYS											
1. METASTASES OF ANGIOSARCOMA		0	0	0	0	0	0	0	0	0	1
2. Nephroblastoma		1	0	0	1	0	0	0	0	0	0
3. Clear cell tumour		0	0	0	0	0	0	0	0	1	0
4. Lipomatous tumour		0	0	1	0	0	0	0	0	0	0
5. Epithelial tumour		0	0	0	0	0	1	0	0	0	0

cont.....

Table 19 cont.

Site and type of tumours	Incidence of tumours									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
THYMUS										
1. Fibrosarcoma	0	1	0	0	0	0	0	0	0	0
2. Reticulum cell sarcoma	0	0	0	0	1	0	1	1	0	0
MESENTERIC LYMPH NODES										
1. Reticulum cell sarcoma	0	0	0	0	1	0	0	0	0	0
SKIN										
1. Squamous cell carcinoma	2	3	3	1	0	0	0	0	1	0
SUBCUTIS										
1. Fibroma	2	1	1	1	1	3	3	1	0	0
2. Fibrosarcoma	0	1	1	0	0	1	1	0	0	0
3. Mesenchymal tumour	0	0	0	0	0	1	0	0	0	0
MUSCLE										
1. Rhabdomyosarcoma	0	0	0	1	1	1	0	0	0	0
SKULL										
1. Osteoma	1	0	0	0	0	0	0	0	0	0
2. Mesenchymal tumour	0	0	0	0	0	0	1	0	0	0
EAR REGION										
1. Adenocarcinoma of unknown origin	0	0	1	0	0	0	0	0	0	0
TESTES										
1. Interstitial cell tumour	3	0	0	1	1					
OVARIES										
1. Theca cell tumour						0	0	1	0	0

cont.....

Table 19 cont.

Site and type of tumours	Incidence of tumours									
	males					females				
	mg VCM/kg body wt					mg VCM/kg body wt				
	0	1.7	5.0	14.1	300	0	1.7	5.0	14.1	300
PRAEPUTIAL GLANDS										
1. Squamous cell carcinoma	0	0	1	0	0	0	0	0	0	0
UTERUS										
1. Adenocarcinoma						6	3	1	1	0
2. Malignant fibroadenomatous tumour						0	1	0	0	0
3. Leiomyoma						0	0	1	0	0
CERVIX										
1. Mesenchymal type of tumour						2	0	1	0	0
2. Adenocarcinoma						0	1	0	0	0
MAMMARY GLANDS										
1. Adenoma	0	0	0	0	0	0	0	0	2	0
2. Fibroadenoma	0	0	0	0	0	21	25	12**	4***	7
3. ADENOCARCINOMA	0	1	0	2	0	3	2	4	7	7
4. Anaplastic carcinoma	0	0	0	0	0	0	0	1	0	0
PRIMARY BLADDER										
1. Epithelial tumour	0	0	1	0	0	0	0	0	0	0

1) Treatment-related tumours or tumours presumably related to VCM exposure are written in capitals.
2) Type and incidence of liver tumours are also presented in table 17; for the sake of completeness they are included in this table too.
3) The figures in this group are not evaluated statistically (see §2.6).
4) In several cases the neoplastic character of the lesion was doubtful.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, according to the Chi-square test.

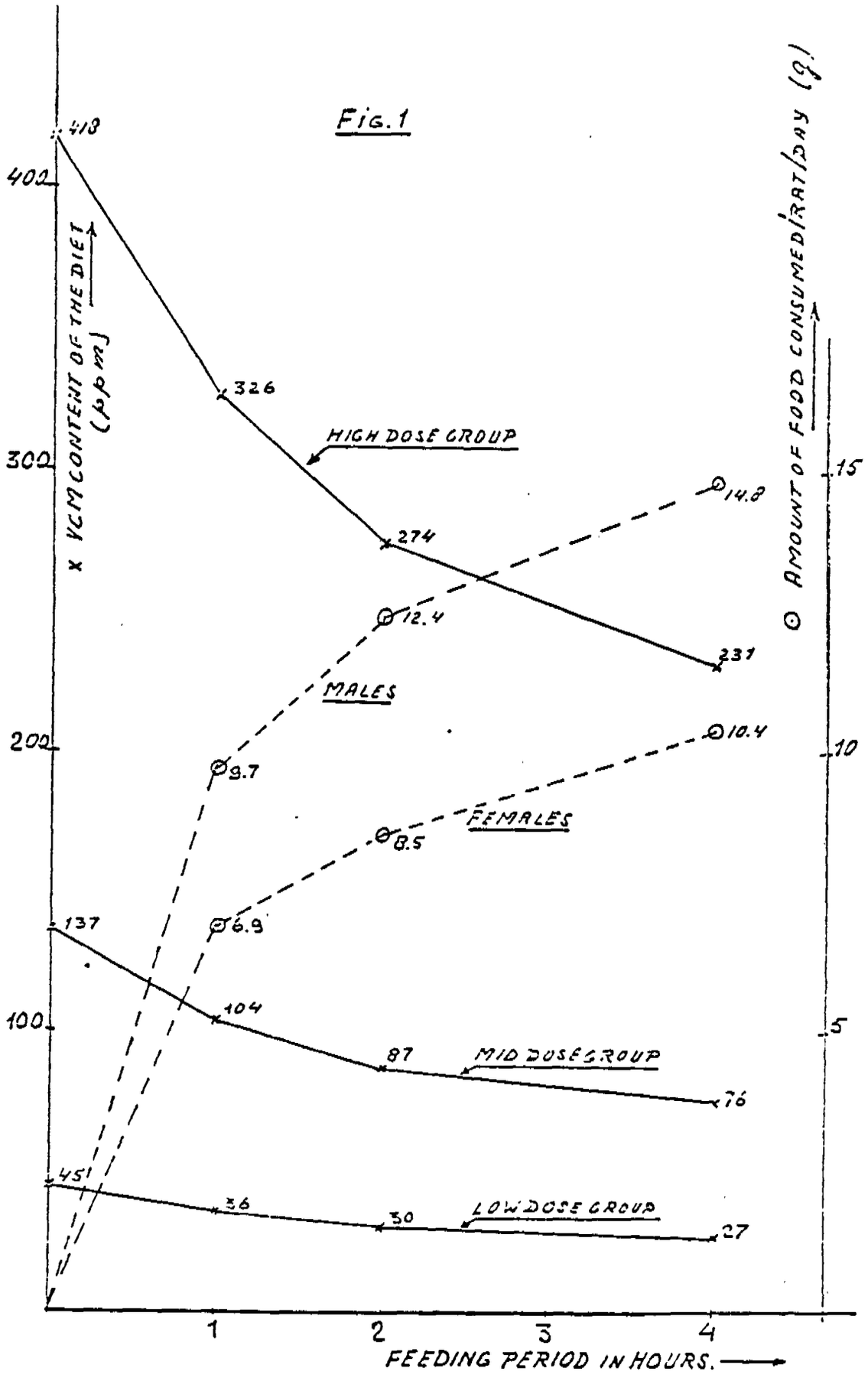


Fig. 1. The average VCM contents of the diets (— x — x —) and the average amounts of food consumed (— o — o —) at different points of time during the 4-hour feeding period.

SL 042022

SL 042023

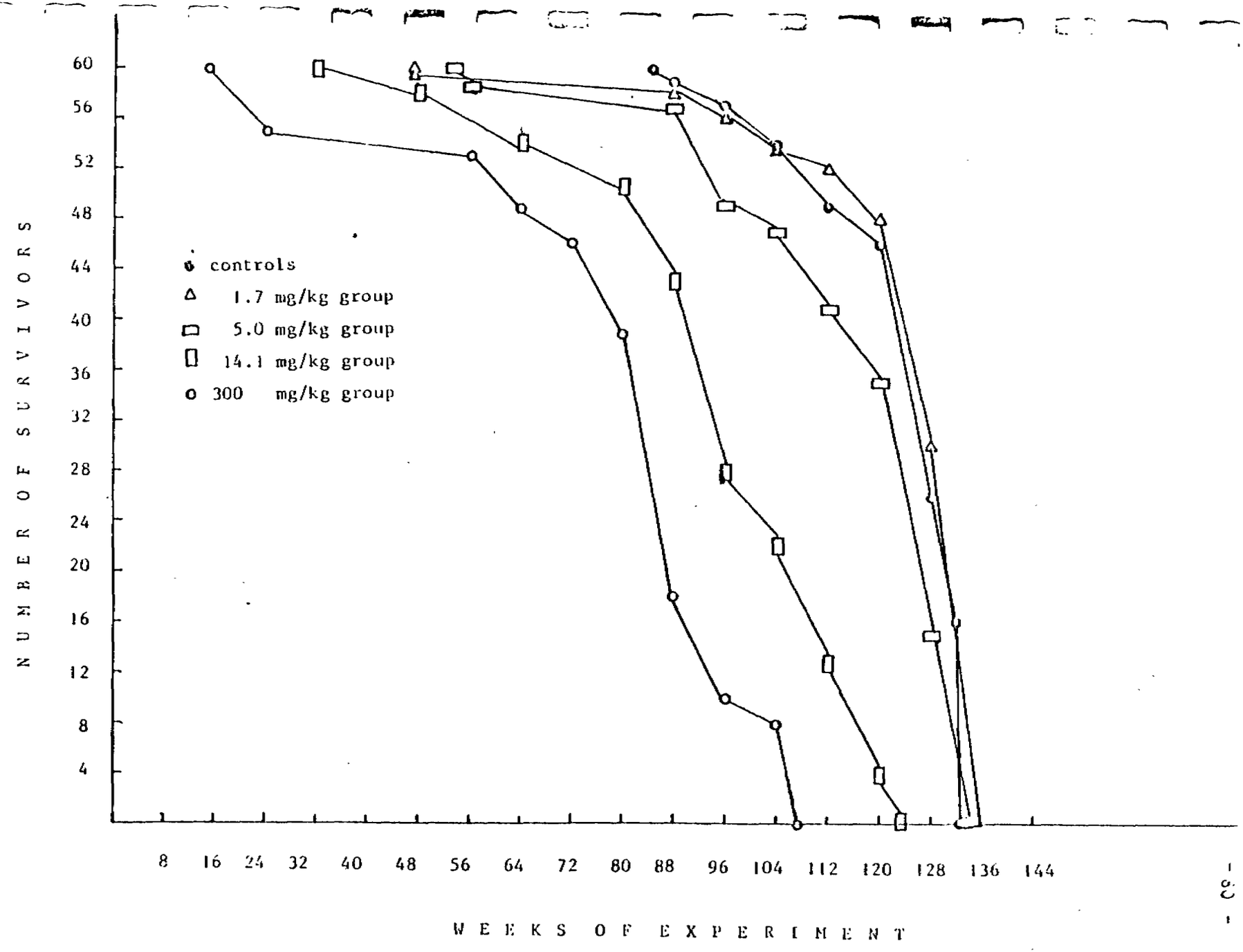
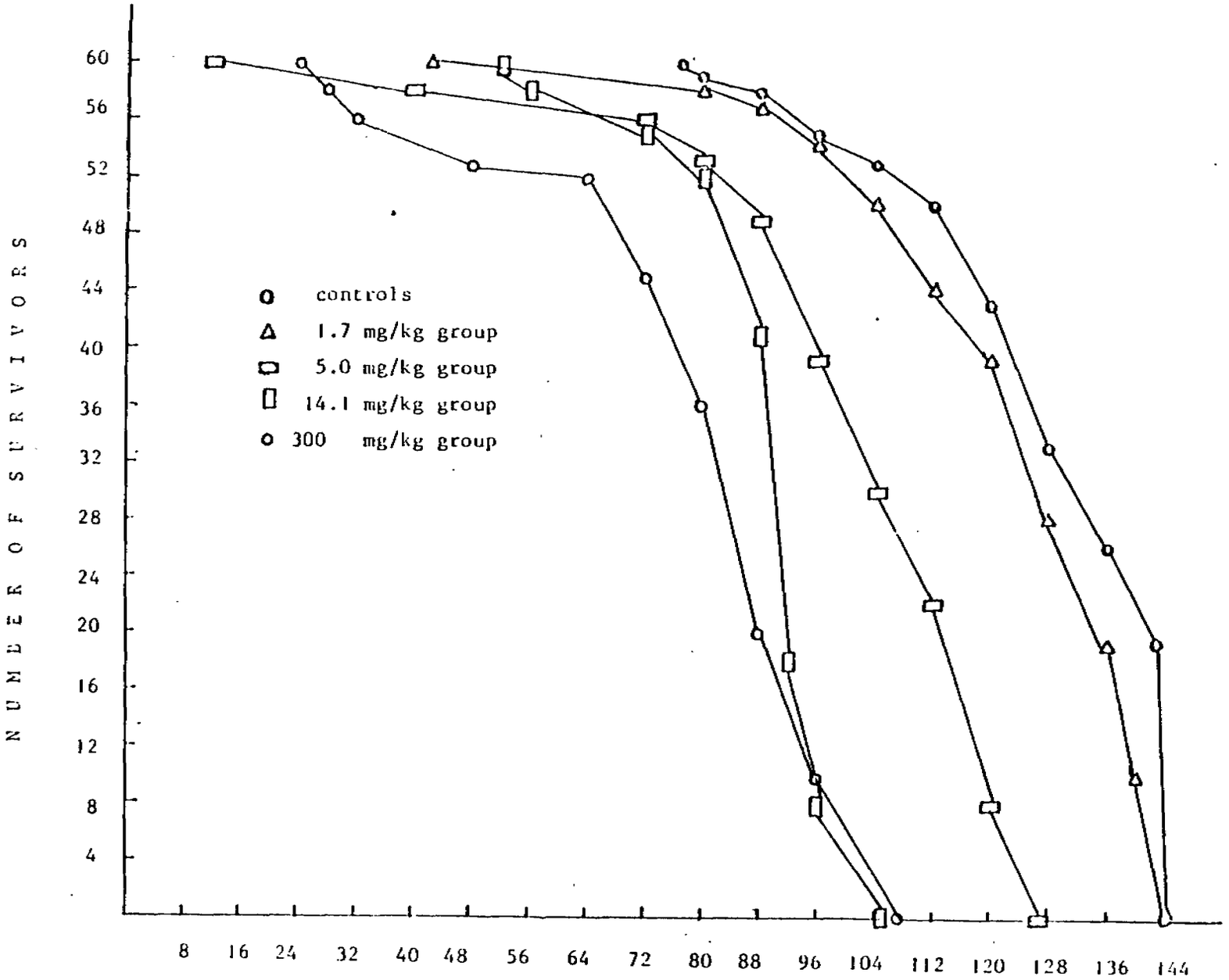


FIG. 2 SURVIVAL RATES OF MALES

SL 042024


NUMBER OF SURVIVORS
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
controls
1.7 mg/kg group
5.0 mg/kg group
14.1 mg/kg group
300 mg/kg group
8 16 24 32 40 48 56 64 72 80 88 96 104 112 120 128 136 144
WEEKS OF EXPERIMENT

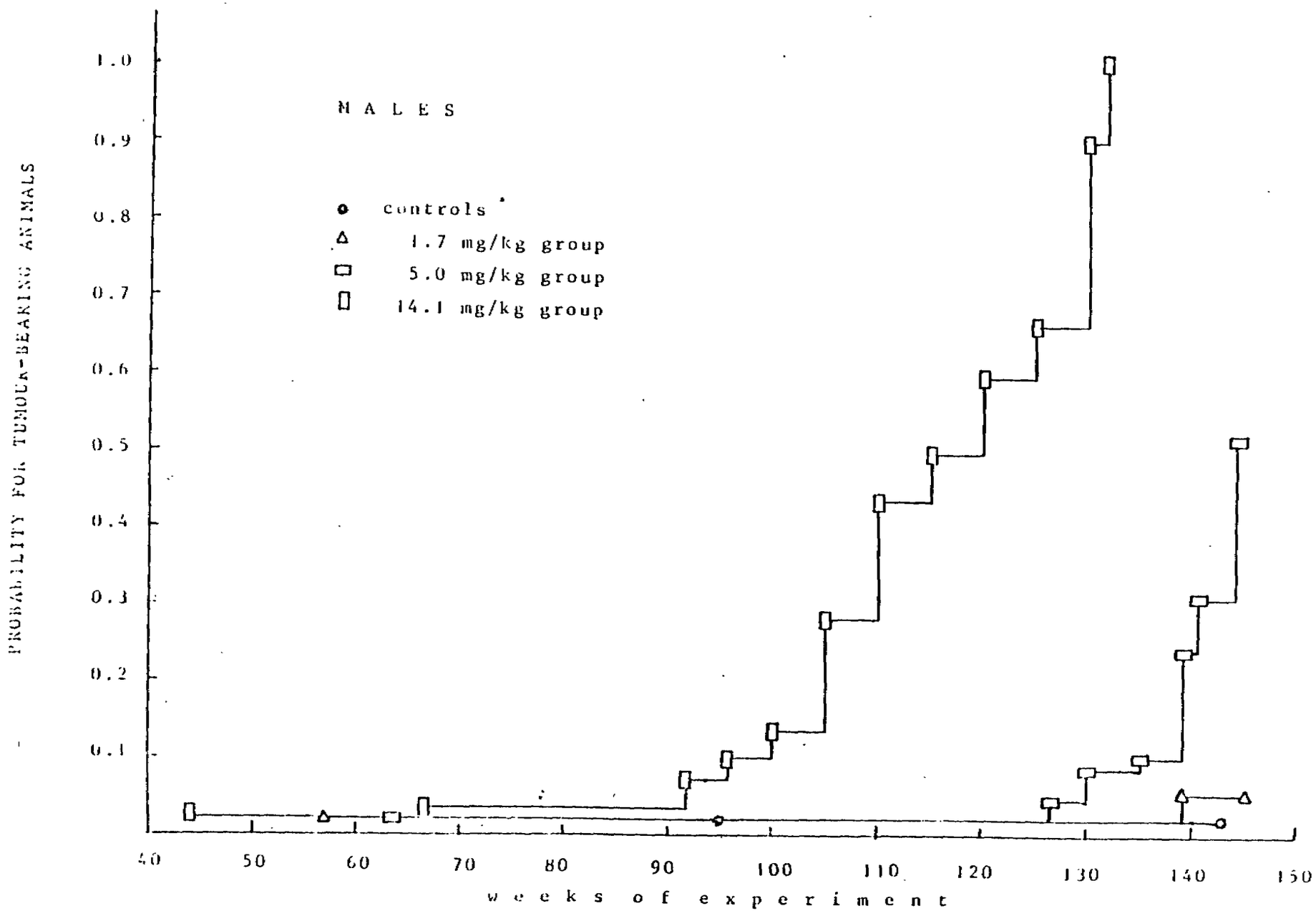


FIG. 4 Probabilities for the observation of a liver tumour (neoplastic nodules, hepatocellular carcinomas and angiosarcomas) at death [illegible]

SL 042026

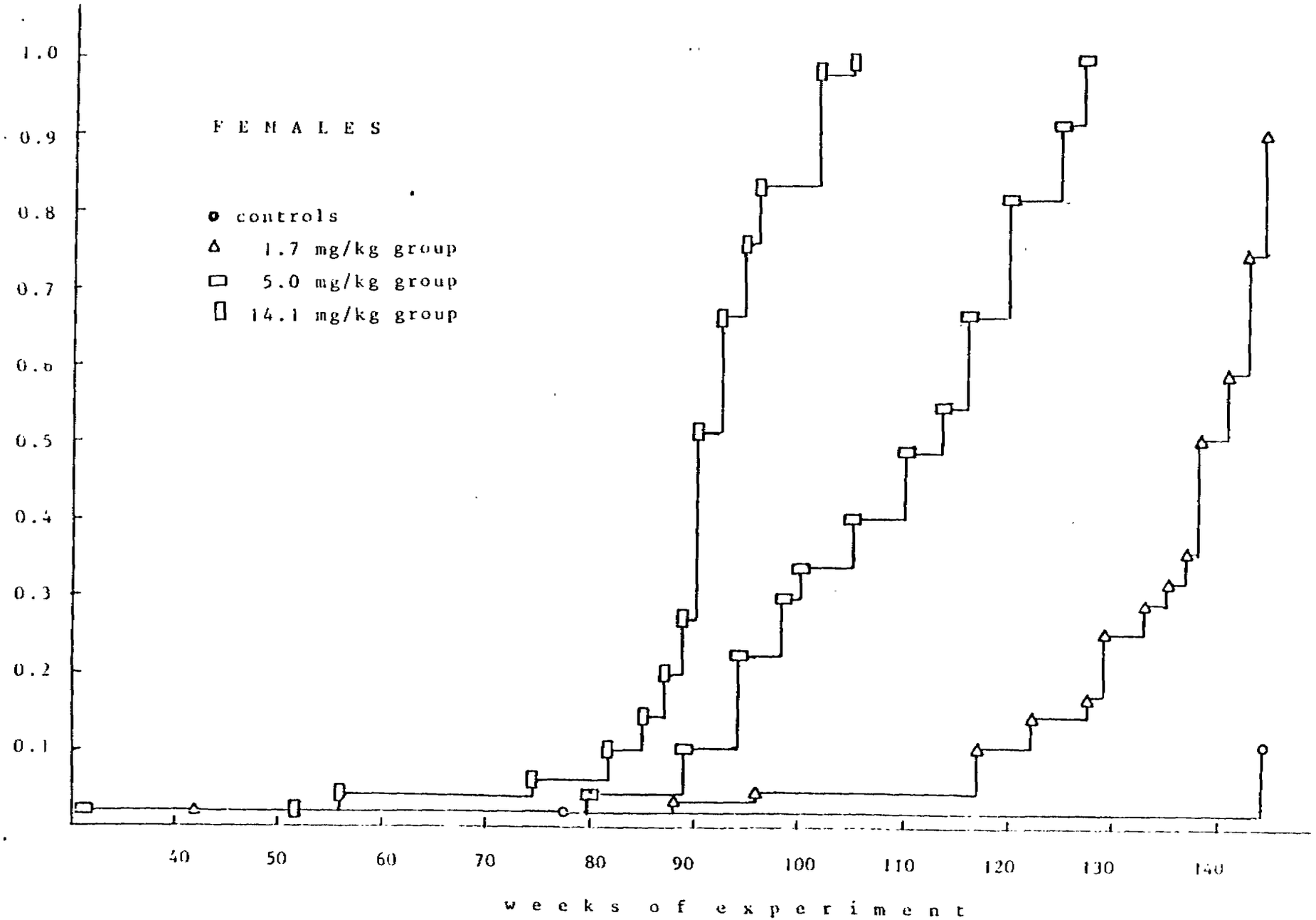


Fig.5 Probabilities for the observation of a liver tumour (neoplastic nodules, hepatocellular carcinomas and angiosarcomas) at death calculated [illegible]

Contaminants in basal diet for rats

Sample of 2000 kg batch produced in July 1976.

lead			1.5 mg/kg
cadmium			0.05 mg/kg
mercury			0.17 mg/kg
tin			-[1]) mg/kg
arsenic			0.2 mg/kg
organo-P-compounds	(dichlorvos, mevinfos, diazanon, malathion, parathion)	<	0.01 mg/kg
organo-Cl-compounds	(HCB, αHCH, β-HCH, γ-HCH, heptachlor, heptachlorepoxide, aldrin, dieldrin, chlordane, DDT's)	<	0.04 mg/kg
carbamates	(as CS_2)		- mg/kg
aflatoxin		<	5 µg/kg
oestrogenic activity (Tiecco-test)			-
3,4-benzpyrene			1 µg/kg
3,4-benzfluoranthene			- µg/kg
indeno (1.2.3.-c.d.) pyrene			- µg/kg
11,12 benzfluoranthene			- µg/kg
fluoranthene			- µg/kg
K-nitrate			370 mg/kg
Na-nitrite			5 mg/kg
dimethylnitrosamine			50 µg/kg [2])
nitrosopyrroiidine			µg/kg
methylethyl-, diethyl-, methylpropyl-, methylbutyl-, dipropylnitrosamin, nitrosopyperidin			- µg/kg
nitrosormorfolin			- µg/kg

[1]) - = not determined

[2]) incorrectly high value caused by DMNA contamination of the solvent dichloromethane

SL 042027

Contaminants in basal diet for rats

Sample of a 2000kg batch produced in February, 1977. Analyses made between March and June 1977.

lead		5.5	mg/kg
cadmium		0.13	mg/kg
mercury		0.08	mg/kg
tin		9	mg/kg
arsenic		0.3	mg/kg
organo-P-compounds	(dichlorvos, mevinfos, diazinon, malathion, parathion)	< 0.01	mg/kg
organo-Cl-compounds	(HCB, αHCH, β-HCH, γ-HCH, heptachlor, heptachlorepoxide, aldrin, dieldrin, chlordane, DDT's)	< 0.04	mg/kg
carbamates		< 0.04	mg/kg
aflatoxin		< 5.0	µg/kg
oestrogenic activity (Tiecco-test)		not detectable	
3,4-benzpyrene		1.7	µg/kg
3,4-benzfluoranthene		2.1	µg/kg
indeno (1.2.3.-c.d.) pyrene		1.2	µg/kg
11,12 benzfluoranthene		0.7	µg/kg
fluoranthene		4.5	µg/kg
K-nitrate		700	mg/kg
Na-nitrite		2	mg/kg
dimethylnitrosamine		90	µg/kg *
nitrosopyrrolidine		2	µg/kg *
methylethyl-, diethyl-, methylpropyl-, methylbutyl-, dipropylnitrosamin, nitrosopyperidin		< 1	µg/kg
nitrosormorfolin		< 5	µg/kg

* Incorrectly high values caused by contaminants in the solvent dichloromethane. With the pure solvent the levels of dimethylnitrosamine and nitrosopyrrolidine were found to be less than one µg/kg

SL 042028

Nutrient composition of basal diet for rats

Analyses of a 2000 kg batch produced in February 1977

moisture	12.7	%
crude protein (N x 6.25)	20.5	%
crude fat	6.2	%
calcium	0.90	%
phosphorus	0.69	%
magnesium	0.13	%
iron	110	ppm
manganese	75	ppm
copper	27	ppm
zinc	45	ppm
cobalt	< 0.1	ppm
chromium	< 0.1	ppm
selenium	--	
vitamin A	12.300	I.U./kg
vitamin D	1.500	I.U./kg
vitamin E	108	mg/kg
carotene	5.4	mg/kg
vitamin K_3	< 1	mg/kg
vitamin B_1	5.9	mg/kg
vitamin B_2	6.4	mg/kg
niacine	68	mg/kg
pantothenic acid	13.9	mg/kg
folic acid	3	mg/kg
choline	1630	mg/kg
biotine	320	µg/kg
vitamin B_{12}	30	µg/kg