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C.I.V.O. VINYLCHLORIDE STUDIES

Re: my letter dated June 3, 1977 (feeding study)

1) Attached you will find a copy of the report on the CIVO - VCM rat inhalation study at 5000 ppm.

Should you have any specific questions regarding this report, please let me know.

2) With respect to the CIVO - VCM rat feeding study, Perry asked some time ago for details on the monitoring of the quantity of VCM actually digested by the animals.
CIVO administers VCM monomer by mixing the feed with PVC of known monomer content as was suggested by me when this study was initiated. We then also discussed the need to carefully monitor the release of monomer from the feed, the avarage rate at which this feed is eaten, and the VCM excreted in the feces. All these parameters are measured and from these the actual VCM intake is calculated. To better control the loss of VCM by evaporation from the feed, the animals are fed only 4 hours per day.

C.I.V.O. people told me that these measurements have shown that the dose levels mentioned in their interim reports, will have to be corrected by no more than about 10%.

If you need more clarification, please let me know.

L.C. RINZEMA

LCR:ms

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REPORT NO. R 5342

Inhalation toxicity of vinyl chloride in rats: A one-year study with a serial killing design

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SUMMARY

1. SPF Wistar rats were exposed to atmospheres containing 0 (controls) or 5000 ppm (0.5 %, v/v) vinyl chloride monomer (VCM), 7 hours/day, 5 days/week, for a period of 52 weeks. After 4, 13, 26 and 52 weeks each time 10 rats/sex/group were killed and subjected to extensive haematological, biochemical and pathological examinations.

2. Slight growth retardation throughout the experimental period and high mortality in the second half of the study were observed in the test group.

3. Some of the haematological indices and biochemical blood parameters were slightly influenced by VCM after an experimental period of 52 weeks only. Blood clotting time was generally slightly shorter in VCM-exposed rats than in controls. There were minor indications of increased potassium contents of the blood serum in VCM-exposed animals during the first half of the test period.

4. The kidneys were adversely affected by VCM as appeared from increases in (a) blood urea nitrogen levels, (b) relative kidney weights and (c) degree of nephrosis.

5. After 52 weeks a slightly increased heart weight and mild focal degeneration of the myocard were noticed in VCM-exposed rats.

6. Increased spleen weights accompanied by increased haematopoietic activity in this organ and slight signs of ischaemia occurred in rats exposed to VCM for 52 weeks.

7. Both parenchymal and sinusoidal cells of the liver were severely damaged by VCM. The major parenchymal changes comprised swelling and malformation of mitochondria, an increased amount of smooth endoplasmic reticulum, necrosis and nuclear and cellular polymorphism of hepatocytes, hyperplasia of hepatocytes and hepatocellular carcinomas. The non-parenchymal alterations included focal dilatation of sinusoids, focal proliferation of atypical sinusoidal cells and multicentric angiosarcomas.

8. In addition to the hepatic neoplasms, primary tumours induced by VCM were found in the nasal cavity, Zymbal's glands, lungs and brain.

9. Main conclusions:

a) VCM is a versatile carcinogen.

b) The present study did not produce obviously suitable parameters to be used as tools for early diagnosing "VCM-disease" in man.

c) Most probably, the effects in rats of (5000 ppm) VCM on the hepatic parenchyma precede those on the hepatic stroma.

Inhalation toxicity of vinyl chloride in rats: A one-year study with a serial killing design

1. INTRODUCTION

Industrial exposure to vinyl chloride monomer (VCM) has been associated with several disorders such as acro-osteolysis, non-neoplastic liver lesions, angiosarcoma of the liver and other types of tumours at various sites (5, 22, 51, 58, 66). Moreover, experimental exposure to VCM by inhalation has been found to result in degenerative and neoplastic changes of the liver and other organs in several animal species (3, 23, 26, 38, 39, 65, 67).

From the point of industrial safety it is important to recognize VCM-effects in plant workers as early as possible (10, 13, 15, 24, 28, 33, 34, 41). Therefore, experiments aimed at detecting the earliest changes attributable to VCM-exposure as well as at studying their further development and consequences, were deemed desirable. Against this background a one-year inhalation study with a serial killing design was conducted with rats repeatedly exposed to VCM at a level as high as 5000 ppm.

2. MATERIAL AND METHODS

2.1. Material

Vinyl chloride monomer (VCM) was obtained from Akzo Chemie, Botlek, Rotterdam, Netherlands, in stainless steel pressure bottles of about 50 litres. Every month a fresh quantity of VCM was delivered.

2.2. Exposure system

From the pressure bottle the VCM-gas was transported at a constant rate by means of stainless steel tubes, a reduction valve and a rotameter to a vertically placed stainless steel/glass cylindrical exposure chamber of 2.5 m^3 capacity, as described by Kruysse et al. (29a). The VCM-flow was mixed with a continuous flow of conditioned air of 18 m^3/h. The inlet was on top of the upper conical part of the chamber. To remove the VCM the exhaust air was passed through a container with 100 l activated carbon, which was partly refreshed every two days.

An exposure chamber, identical to the test chamber, contained the control animals. Through this chamber only filtered and conditioned air was passed at a similar rate.

2.3. Animals, housing, treatment and feeding

One hundred and twenty four male and 124 female, newly weaned, albino Wistar rats obtained from the randomly bred SPF-colony of the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, Netherlands, were evenly distributed according to sex and body weight over two inhalation chambers, one control chamber for exposure to air and the other for exposure to VCM. VCM was dosed at an average concentration of 4950 ppm (12,800 mg/m^3), 7 hours/day, 5 days/week, for a period of 52 weeks. The design level of 5000 ppm VCM is used further in this report.

The rats were housed in tinned, wire-screen cages (two/cage), which were suspended in a heptagonal central frame, rotatable round the vertical axis of the inhalation chamber. The temperature in the chambers was 24 $\pm$ 1 °C, the relative humidity of the air inside 60 $\pm$ 5 %.

The animals were fed the Institute's stock diet having the following percentage composition: yellow maize 29.7, whole wheat 36.0, soya bean oil meal 11.0, fish meal 7.0, meat scraps 4.0, dried whey 2.0, brewer's yeast 3.0, grass meal 3.0, steamed bone meal 0.4, trace mineralized salt 0.5, vitamin preparations 0.4 and soya bean oil 3.0.

Fresh batches of the diet were prepared once a forthight and stored at room temperature. Diet and tap water were constantly available.

2.4. Experimental design and conduct

2.4.1. Body weights and killing schedule

Individual body weights were recorded once a week during the first month, once a fortnight during the second and third month, and once monthly thereafter.

After 4, 13, 26 and 52 weeks, 10 rats/sex/group were killed by either exsanguination from the abdominal aorta after light ether anaesthesia or perfusion with 2.5 % glutaraldehyde solution through the portal vein after Nembutal anaesthesia, and subjected to extensive examinations described in the following.

2.4.2. Haematology

At weeks 4, 13, 26 and 52, blood samples were collected from the tip of the tail of ten rats of each sex from the control and test group. All blood samples were examined for the following parameters: haemoglobin concentration (Hb), by cyanmethaemoglobin method of Van Kampen and Zijlstra (25); packed cell volume, as microhaematocrit; blood clotting time using Normotest reagents from Nyogaard and Co; Oslo, Norway; red blood cell, white blood cell and thrombocyte counts by Coulter Counter; differential white cell count by direct visual count of smear after May-Grünwald-Giemsa staining, according to Gorter and De Graaff (21). Counts are recorded under the following categories: L = lymphocytes; N = neutrophils; M = monocytes and E = eosinophils.

2.4.3. Clinical chemistry

Fasting blood glucose and blood urea nitrogen (BUN) were determined at weeks 4, 13, 26 and 52 using the Technicon Auto Analyzer method N-9a for glucose and the automated phenazone/diacetyl monoxime technique of Ceriotti and Spandrio (9) for urea. The analyses were conducted upon blood from the tip of the tail of ten rats of each group and sex after overnight fasting of the rats.

At weeks 4, 13, 26 and 52, samples of blood were collected at autopsy from ten rats of each group and sex. The following measurements were made in the serum after centrifugation at 3000 rpm for 20 minutes: lactic dehydrogenase (LDH) according to the method of Wróblewski and La Duc (71), using test reagents of Boehringer, Mannheim, W-Germany; alkaline phosphatase (SAP), by the method of Bessey et al. (6), using a Technicon Auto Analyzer; glutamic-oxalacetic transaminase (SGOT) and glutamic-pyruvic transaminase (SGPT), according to the method of Reitman and Frankel (54), using a Technicon Auto Analyzer; total protein (TSP), by biuret reaction; albumin according to the method of De Leeuw-Israel et al. (31); serum protein pattern by the agar-gel electrophoretic method on microscope slides of Wieme (69), staining with nigrosin and quantitative evaluation of the electropherograms by transmission densitometry of the strips; serum α-fetoprotein levels according to a radio immuno assay (6b); the serum electrolytes sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg), according to the method of Paschen and Fucks (48), and Cl according to a coulometric method.

2.4.4. Urinalyses

Individual urine samples were collected from ten rats of each group and sex, at weeks 4, 13, 26 and 52, 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); uric acid (21); glutamic-oxalacetic transaminase activity (UGOT), according to the method of Reitman and Frankel (54) using reagents from Baker Chemicals N.V., Deventer, Netherlands.

In pooled urine samples, quantitative measurements were made of pH, protein, sugar, occult blood, ketones using Labstix from Ames Laboratories and microscopy of sperm deposit - after centrifugation at 3000 rpm for 3 minutes. Deposits were examined for: erythrocytes, leucocytes, epithelial cells, amorph substances, crystals, phosphate crystals, casts, bacteria, worm eggs and sperm cells. The following grading system was used: - = negative; ± = minimal; + = slight; ++ = moderate; +++ = high; ++++ = very high.

2.4.5. Liver function tests

2.4.5.1. BSP-extinction test

Bromosulfophthalein (BSP) was injected iv (25 mg/kg body weight) and after exactly ten minutes blood was collected from the orbital sinuses. The BSP-concentration in the serum was determined colorimetrically by measuring the absorbance at 580 nm. Five rats/sex/group were used.

2.4.5.2. Barbiturate sleeping time

Sodium pentobarbital was injected ip (15-50 mg/kg body weight) and sleeping time was recorded according to Balazs and Grice (1) in ten rats/sex/group.

2.4.6. Kidney function test

The phenol red excretion test was carried out according to a modification of the procedure described by Sharrat and Frazer (59). Each rat was given an intramuscular injection of 0.1 mg of phenol red (in saline) per kg body weight. The total urine produced in 60 minutes was collected, and the concentration of phenol red was estimated colorimetrically by measuring the absorbance at 558 nm. Ten rats/sex/group were used.

2.4.7. Pathology

The animals killed after 4, 13, 26 and 52 weeks were autopsied and subjected to a careful gross examination. The heart, kidneys, liver, spleen, brain, gonads, thymus, pituitary, thyroid, adrenals and lungs (with trachea and larynx) were weighed. Samples of the organs weighed and of the following organs and tissues were preserved in 10 % buffered formalin: pancreas, genital organs, skeletal muscle, spinal cord, urinary bladder, peripheral nerve, salivary glands, mesenteric and axillary lymph nodes, bone marrow (sternum), esophagus, stomach, small and large intestines, skin, bone, trachea, larynx, nasal cavity, aorta, exorbital lachrymal glands, Hardsrian gland, ceruminous glands and other organs showing gross lesions.

Organs and tissues to be studied microscopically were processed through paraffin, sectioned at 5 µm (nasal cavity, larynx, trachea, lungs, brain, thyroid, adrenals, pituitary and liver at two, three or four levels) and stained with haematoxylin and eosin.

Of all control and test rats killed at week 52, all tissues preserved were examined histologically. The organs of the rats killed after 26 weeks that were examined microscopically comprised liver, kidneys, ceruminous glands, lungs, brain, bone, nasal cavity, spleen and heart. Microscopic examination of the animals killed after 4 or 13 weeks was restricted to liver, kidneys, ceruminous glands, lungs, bone and brain.

Animals found dead or killed in moribund condition, and all controls killed in week 52 when all VCM-exposed animals were dead, were also autopsied, and a wide range of tissues was fixed in formalin if autolysis was not too advanced. Histological examination of these animals principally concerned all organs showing gross lesions, and moreover, of the VCM-exposed animals, those organs expected to harbour VCM-related neoplastic lesions of microscopic size such as the lungs, brain, nasal cavity, kidneys and liver.

2.4.8. Enzyme histochemistry of the liver

The following enzyme histochemical reactions were carried out on cryostat sections of liver specimen (frozen in isopentane and stored at -70 °C) from 4 rats/sex/group killed in week 52: alkaline phosphatase (19), acid phosphatase (2, 20), adenosine monophosphatase and glucose-6-phosphatase (68).

2.4.9. Electron microscopy of the liver

After 4, 13, 26 and 52 weeks each time liver tissue of 2 rats/sex/group was collected for electron microscopical examination. Liver samples taken after 4 weeks were fixed by immersion, those collected at later points of time 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) and contained 1 % sucrose. The perfusion flow was 5 ml/100 g rat/minute. Within 2 minutes after switching over from perfusing with 0.9 % NaCl solution to perfusing with the fixative, the liver was removed from the body, and a few slices from the left lobe were immediately cut into 1 mm^3 blocks, which were stored in the fixative overnight at 4 °C. Thereafter, 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 acetone/water mixtures was followed by embedding in Epon 812. One micron sections were stained with Paragon, and ultra-thin sections were stained with uranyl acetate and lead citrate, and viewed with a Philips EM200 or EM201G electron microscope at 60 kV.

2.4.10. Statistical analysis

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.

3. RESULTS

3.1. Behaviour, mortality and growth

During the first half of the experimental period the behaviour of the test animals did not differ from that of the controls. No deaths occurred during the first half year (Table 1). The first animal that died was a male of the test group. It was found dead in week 33 and the presumable cause of death was intraabdominal haemorrhage. Thereafter, mortality among VCM-exposed rats gradually increased, and in week 52 only 9 males and ten females of the test group were still alive. Several animals that died or were killed *in extremis* lost body weight and became lethargic a few weeks prior to death. Some of the lethargic animals were no longer able to walk, stopped eating and drinking and became filthy. All of them appeared to have in their brain either a

primary tumour or metastases from tumours in other organs. The controls remained in good shape, except for one lethargic animal which was killed in week 50 and was found to have a swollen degenerated liver, probably due to leukaemic infiltrates.

Mean body weights in the test group were significantly decreased throughout the experimental period, the differences with the controls being more marked in males than in females (Table 2).

3.2. Haematology

Mean haematological indices are given in Table 3.

Haemoglobin content, packed cell volume and the number of erythrocytes were slightly decreased and the total number of leucocytes were somewhat increased in VCM-exposed males and females at week 52, although the differences with the controls were not always statistically significant.

Blood clotting time in test animals was lower than in controls at all intervals examined, the differences with the controls being statistically significant in males at weeks 4 and 52 only.

The slight increase in packed cell volume and erythrocyte counts in males at weeks 4 and 26 respectively, the slight increase in thrombocyte counts in males at week 26 and some slight deviations in differential counts in females at week 26 and in males at week 52 were not consistent and are, therefore, considered of no toxicological significance.

3.3. Clinical chemistry

Mean biochemical blood values are given in Table 4.

Blood urea nitrogen levels were slightly increased in the test group in females at week 26 and in males and females at week 52.

Albumin and γ-globulin levels were slightly decreased in the test group in both sexes at week 52 only.

Alpha-fetoprotein levels were slightly increased in the test group after 26 and 52 weeks, the differences with the controls being statistically significant after 52 weeks only (Table 4). A markedly elevated level of this protein indicating the presence of a α-fetoprotein-producing tumour, was not found in any of the animals.

The potassium content of the blood serum was somewhat higher in test animals than in controls during the first half year of the experimental period (Table 4).

Other slight deviations in the test group as compared to the controls occurred only at one of the intervals and in only one of the sexes. Therefore, they are not considered to be of toxicological importance.

3.4. Urine analyses

There were no changes in the composition of the urine that could be ascribed to treatment (Table 5).

Specific gravity values and uric acid levels were decreased, whereas the volume of the urine produced and UGOT-values were increased in both males and females of the test group at week 52 only (Table 6). However, the UGOT-values are well within the normal range and well below those considered pathological.

The increase in phenol red excretion observed in males of the test group at week 13 is probably a result of the low excretion found in the controls at that time (Table 6).

The slight decrease in volume and increase in uric acid in males t week 13 were single findings and probably fortuitous and unrelated to treatment (Table 6).

3.5. Liver function tests

Mean results of the liver function tests are given in Table 7.

BSP-retention was decreased in the test group at weeks 13, 26 and 52, but slightly increased in females at week 4.

Sleeping time showed no consistent differences between the test group and the controls, though it was definitely increased in VCM-exposed males at week 4.

3.6. Organ weights

Mean relative organ weights are shown in Table 8.

In the test group, the relative weights of the liver and kidneys were nearly always significantly increased in both sexes at weeks 13, 26 and 52 and those of the spleen at weeks 26 and 52.

The relative weights of the heart and lungs were slightly increased in the test group at week 52, but the differences were statistically significant in only one of the sexes.

The increases in the relative weights of the brain and the testicles in the test group at some intervals is ascribed to the lower body weights

of the VCM-exposed rats, because of the well-known inverse correlation between body weight and relative brain and testicle weights (16).

Other changes in relative organ weights between the test group and the controls were not consistent, and are not regarded as being of toxicological significance.

3.7. Pathology

3.7.1. Gross examination

Gross autopsy findings in animals killed after 4, 13 or 26 weeks were essentially negative, except for a wart-like growth, measuring 2 cm in diameter in the area of the left ear of a VCM-exposed female killed after 26 weeks. Upon microscopy, this tissue mass turned out to be a highly keratinized squamous cell carcinoma arising from the left ceruminous gland (Zymbal gland).

A variety of gross lesions related to VCM-exposure were seen in rats that died, or were killed either in moribund condition or at week 52. The liver of about 50 % of the animals of the test group contained nodule-like processes widely varying in size (up to 3 cm in diameter), appearance and consistency. Some of them were solid and pale, others cystic, haemorrhagic and necrotic. Adhesions between an affected liver and other abdominal organs were not uncommon. The abdominal cavity of several rats found dead contained blood, most probably derived from ruptured cyst-like structure in the liver.

In the area of the right or left ear wart-like growths were seen in six males and four females of the test group.

In the lungs small haemorrhagic nodules (2 to 6 mm in diameter) were found in a few rats exposed to VCM (2 males and 3 females). The nodules were nearly always located at the edge of a lobe. In a few more animals of the test group (5 males and 5 females) the lungs exhibited small greyish or haemorrhagic areas or were heavy and oedematous. In some animals an entire lobe was consolidated, which was most probably due to bronchopneumonia.

A mucous and pus-like nasal discharge was seen in a few rats of the test group. Some other test animals had a deformed nose due to a small focal swelling suggestive of a tumourous growth in the nasal cavity.

None of the above changes were observed in controls.

Randomly distributed gross changes unrelated to VCM-exposure included among others greenish kidneys with a granular surface, pale

livers, ovarian cysts, white patches in the exorbital lachrymal glands, enlarged axillary lymph nodes, swollen uterine horns and testicular atrophy.

3.7.2. Histopathology of rats killed after 4, 13, 26 and 52 weeks

Site, type and incidence of the histopathological changes observed in animals killed after 4, 13, 26 and 52 weeks are shown in Tables 9, 10, 11 and 12 respectively.

From Tables 9 and 10 it appears that after 4 and 13 weeks no treatment-related alterations were observed, with the possible exception of a few small foci of clear cells in the liver of one male test animal killed after 13 weeks. The relevance of this minimal liver change might be questioned. However, it is beyond any doubt that after 26 and 52 weeks (Tables 11 and 12) similar but much more pronounced hepatic changes occurred nearly exclusively in VCM-exposed rats. In addition, after 26 and 52 weeks a number of rats treated with VCM had basophilic foci in their livers. Cellular and nuclear polymorphism of hepatocytes were invariably present in test animals killed after 52 weeks. Nodules or tumours arising from hepatocytes - not yet found after an experimental period of 26 weeks - did occur in several VCM-exposed rats killed after 52 weeks. One of them had a hyperplastic liver cell nodule with atypia and two animals were found to have hepatocellular carcinomas (Table 12). Moreover, neoplasms in the liver originating from sinusoidal cells were encountered in nine out of the nineteen rats examined after 52 weeks. These tumours were classified as angiosarcomas, though in three cases anaplastic carcinoma could not be excluded. Many of the rats bearing these "sinusoidal cell tumours" also showed focal distension of sinusoids and focal proliferations of relatively normal or atypical sinusoidal cells. Extensive areas of liver necrosis and large cyst-like spaces filled with haemorrhagic fluid and cellular débris were seen in one male of the test group. In addition, focal proliferation of atypical sinusoidal cells not (yet) considered to be neoplastic in nature was seen in one male and one female test animal not bearing a tumour of the liver.

The first tumour observed was a squamous cell carcinoma of the ceruminous gland (Zymbal gland) in a female test animal killed at week 26 (Table 11). This type of tumour was also found in five out of the nineteen test animals killed after 52 weeks. In addition, the ceruminous glands of three other animals killed at that time exhibited epithelial hyperplasia.

Pulmonary lesions attributable to treatment were observed only in animals killed after 52 weeks. They consisted of haemorrhages, adenomatous hyperplasia of bronchiolar epithelium (adenomatoid lesion), focal increased cellularity of interalveolar septa, metastases of liver angiosarcomas, and one small papillary adenoma (Table 12).

After 52 weeks tubular nephrosis was found in all males and in several females. Degree and incidence of the lesion were clearly higher in the test group than in the controls. In addition, one of the kidneys of a VCM-exposed male rat killed after 52 weeks contained a small focus of atypical hyperplastic tubular epithelium (Table 12). No such renal effects of VCM were encountered at the earlier stages of the experiment.

Several rats exposed to VCM for 52 weeks showed an increased haematopoietic activity in the spleen and minimal degeneration of the myocard (Table 12). After an experimental period of 26 weeks no such splenic or cardiac changes were found.

A remarkable finding was the occurrence of hyperplastic and neoplastic alterations of the olfactory epithelium in the nasal cavity of several test animals killed after 52 weeks (Table 12). The hyperplastic changes were seen as proliferations of atypical bas l cells and cells of Bowman's glands. The tumours penetrated the underlying tissues and were classified as highly infiltrative carcinomas. In several cases the tumours destroyed the nasal bones over large areas and grew outside the nasal cavity.

Other organs examined microscopically did not exhibit lesions that could be attributed to VCM-exposure.

3.7.3. Total incidence and morphology of tumours

Location, type and total incidence of tumours are presented in Table 13. No tumours were found in the control group apart from a granulosa cell tumour in the left ovary of one female killed in week 53. This low tumour incidence in the control group is perfectly

normal, because, when killed, the animals were at most 13 months old and "spontaneous" tumour incidences of some extent generally do not occur until at the age of 18-24 months.

In the livers of many test animals that died or were killed after an experimental period of 52 weeks, extensive areas of necrosis were found. In several of them cyst-like spaces occurred that were filled with haemorrhagic and necrotic material and occasionally surrounded by a layer of connective tissue. In a number of these rats no indications were found for a neoplastic response in the liver. In others, however, the liver contained large and highly malignant tumours. Difficulties in differentiating between reactive angiofibroblastic hyperplasia and neoplasia of sinusoidal cells were occasionally met, in particular in animals that were somewhat autolytic. However, a lesion was only indicated as a tumour when the diagnosis was free from any reasonable doubt. Apart from hyperplastic nodules with atypia, two major types of tumours were found in the liver: hepatocellular carcinoma and angiosarcoma (Table 13). The liver cell lesions were classified according to Squire and Levitt (61), except for the use of the circumscription "hyperplastic nodules with atypia" instead of "neoplastic nodules". The morphology of the angiosarcomas needs some further description. The tumours were multicentric and often contained fairly large foci of necrosis, haemorrhages and thrombus-like material. In most of the tumours the neoplastic cells tended to line spaces, the wall of which was thickened by fibrous tissue. The neoplastic cells were polymorphic and had hyperchromatic nuclei. The amount of fibrous connective tissue in the neoplasms varied widely. The direct extension of some of the more angiomatous tumours to the adjacent liver tissue was seen to occur in a destructive way, whereas other more solid tumours extended to the adjacent tissue by infiltration between preserved liver cords. In view of their growth pattern, the tumours were considered to have developed from sinusoidal cells, most probably endothelial cells, though other sinusoidal cells (Kupffer-cells, fat storing cells, fibroblasts, pit-cells) could not be fully excluded. In five cases an unequivocal classification of the tumours could not be given, because a clear differentiation between "sinusoidal cell sarcoma" and anaplastic (hepatocellular) carcinoma was not possible.

Lung metastases were found in eleven rats bearing primary liver tumours, which were diagnosed either angiosarcoma or anaplastic carcinoma.

Most of the neoplasms found in the ceruminous glands (Zymbal glands) were well-differentiated, highly keratinized squamous cell carcinomas (Table 13). In two cases no histological evidence was obtained of the tumours infiltrating the underlying tissues. Hence, these neoplasms were classified as papillomas. In one instance, part of a malignant tumour presented a glandular pattern. It was, therefore, called an adeno-squamous carcinoma. No metastases of Zymbal gland tumours were observed.

Primary lung tumours were encountered in two VCM-exposed rats (Table 13). One of them had a small papillary adenoma and the other showed multiple pulmonary nodules consisting of a mesenchymal type of cells. Haemorrhages and necrotic areas were common, and an angiomatous pattern was occasionally seen. The tumour had metastasized to the brain and kidneys, and was listed as a malignant mesenchymal tumour, possibly a poorly differentiated angiosarcoma.

Only one primary brain tumour was found (Table 13). It occurred in the anterior part of the cerebrum of a male test animal killed in week 45. The tumour, which was not detected at gross examination, appeared to be in direct contact with one of the ventricles and was seen to originate from normal ependyma. Rosettes and pseudorosettes were characteristic features, and a vascular reaction was visible in the adjacent tissue. Mitotic figures and isolated-cell necrosis were excessive. The tumour was classified as malignant ependymoma.

Tumours found in two other test animals were located in the same part of the brain and morphologically resembled the above ependymoma although true rosettes were rare and a vascular reaction was absent. Since one of these animals had a highly malignant carcinoma of the olfactory epithelium in the nasal cavity which was morphologically similar to the brain tumour, and the nasal cavity of the other animal could not be examined histologically because the nose was lost, the tumours in the brain were considered to represent most probably metastases rather than primary brain tumours. In addition, six other VCM-exposed rats exhibited tumours of the anterior part of the brain which were easily recognized as metastases of nasal cavity

carcinomas. Against this background, some hesitation might be justified as to the correctness of the above diagnosis malignant ependymoma, though in this case the histological evidence of primary brain tumour was fairly convincing, and, moreover, no tumour was found in the nasal cavity.

In the nasal cavity of 20 VCM-exposed rats malignant tumours were found (Table 13). Nearly all of these rats also showed atypical hyperplasia of the basal cells of the olfactory epithelium as well as of Bowman's glands. In addition, this form of hyperplasia was seen in many other rats of the test group. The tumours often destroyed parts of the turbinals and occasionally extended to organs outside the nasal cavity, e.g. the brain. Eighteen of the neoplasms were diagnosed as carcinomas originating in the olfactory epithelium. They predominantly consisted of either relatively small cells resembling basal cells or larger basophilic cuboidal cells with vesicular nuclei suggestive of cells of Bowman's glands. Some of the tumours having mainly solid structures, resembled basal cell carcinomas, whereas others showed cord- and gland-like formations Mitotic figures were always abundant. Areas of necrosis and isolated-cell necrosis were common.

In one of the nasal cavity tumours sarcomatous structures predominated but epithelial elements were also present. Therefore, this tumour was classified as a carcino-sarcoma. Another tumour contained columnar cells arranged in rosettes occupying the major portion of the neoplasm. The spaces lined by the tall cells often contained eosinophilic material. The histological appearance of the tumour was highly suggestive of esthesioneuroepithelioma, though a pure epithelial origin of the tumour could not be excluded.

3.8. Enzyme histochemistry of the liver

The enzyme-histochemical examinations were confined to animals killed after 52 weeks.

In VCM-exposed rats an increased activity of acid phosphatase was seen in Kupffer-cells (or other sinusoidal cells) located in the periphery of the hepatic lobules. In areas where the sinusoids were distended, sinusoidal cells did not show any activity of acid phosphatase, indicating Kupffer-cells being either absent or inactive with

regard to this enzyme. Moreover, "foci of cellular alterations" or hyperplastic nodules exhibited much less pericanalicular acid phosphatase activity than normal liver tissue. In comparison with normal liver cells the hepatocytes in these foci of altered liver tissue showed a reduced glucose-6-phosphatase activity. It was interesting to find a strong sinusoidal activity of alkaline phosphatase within the foci of cellular alterations. An increased sinusoidal activity of this enzyme was also observed in areas showing distended sinusoids. No difference in adenosine monophosphatase activity was found between livers of test and control animals.

3.9. Electron microscopy of the liver

Upon light microscopy of semi-thin sections, small foci of hepatocytes, containing a finely "vacuolized" cytoplasm, were found in most of the VCM-exposed rats killed after 4, 26 and 52 weeks. No such "vacuolized" liver cells were encountered in any of the controls or in test animals killed after 13 weeks. In two of the VCM-exposed rats killed after 52 weeks, the "vacuoles" were more numerous and larger than in all other cases.

Ultrastructurally, the "vacuoles" appeared to represent swollen mitochondria with a pale augmented matrix and relatively short cristae arranged radially and restricted to the periphery, thus leaving a fairly large central area free from cristae. Hepatocytes with swollen mitochondria occasionally were found to contain myelin-like figures localized in lipid droplets or mitochondria.

After 13 weeks no swollen mitochondria were seen, but cup-shaped mitochondria accompanied by short stacks of rough endoplasmic reticulum (RER) were occasionally observed in a limited number of hepatocytes fairly poor in cytoplasmic organelles.

After an experimental period of 26 weeks swollen mitochondria were again observed. They were often empty or deprived of cristae. Irregularly shaped mitochondria with a heterogeneous matrix (light- and dark-grey areas) was a frequent finding in hepatocytes not containing swollen mitochondria. In these hepatocytes the RER consisted of olearly shortened packets of wavy lamellae, and mitochondria were often surrounded by single cisternae of RER. Occasionally, small areas were found in the liver not showing the normal hepatic architecture. The liver cells in these regions were joined together in an irregular

way. There was a remarkable cell demarcation, invariably seen as a thin layer of condensed, somewhat filamentous cytoplasm adjacent to the plasma membrane. Bile canaliculi were nearly always present, but sinusoids or sinusoid-like spaces occurred only sporadically in these areas. The amount of tubular smooth endoplasmic reticulum (SER) was increased, whereas the amount of RER, generally occurring as short stacks around the mitochondria, was decreased. In some cases, liver cells were filled with whorl-like bands of tubular SER.

The liver changes found after 52 weeks were similar to those seen after 26 weeks, but generally much more pronounced. Remarkable findings were cells tightly packed with swollen mitochondria often filled with flocky material, RER showing loss of ribosomes, absence of glycogen particles and accumulations of free ribosomes.

4. DISCUSSION

In the present study with rats repeatedly exposed to VCM at a concentration as high as 5000 ppm (0.5 %, v/v) a great number of effects were found that could be attributed to the test substance. Several of these effects, such as mortality, liver changes and tumours in various organs, were not unexpected and confirmed the results obtained by other investigators in studies with several animal species (see Haley, 22, for a review of the toxicological data).

The growth retardation visible in the test group already after a few days, and persisting throughout the experimental period may be an indication that the VCM-exposed rats did not feel quite fit from the start of the experiment, though no abnormal behaviour was seen in these rats. Growth retardation has also been seen in guinea pigs exposed to 10 % VCM, 2hr/day, for a period of 92 days (53). On the other hand, Viola et al. (67) observed no growth reduction and no behavioural disturbance in rats exposed to 3 % VCM (4 hr/day, 5 days/week) for 12 months. In addition, Maltoni and Lefemine (37) and Maltoni et al. (39, 40) did not report an effect on growth in their long-term studies with rats, mice and hamsters exposed to VCM-levels up to 1 % (10000 ppm).

Increased numbers of erythrocytes and decreased numbers of white blood cells have been found in rats following repeated exposure (8 hr/day) to 5 and 2 % VCM for 19 and 92 days respectively (32). The investigators did not consider these changes the manifestation of a specific toxic

action of VCM, because there were no histopathological alterations that could be connected with the abnormal numbers of red and white blood cells. The same seems to hold for the slight deviations in haemoglobin content, packed cell volume and the number of red and white blood cells found in the present study at several stages, though the increase in both the weight and the haematopoietic activity of the spleen observed after 52 weeks may be related to the haematological alterations occurring at that time. The toxicological significance of these splenic changes is not clear, but in this respect it may be mentioned that splenomegaly has been observed in workers chronically exposed to VCM (14, 17, 30, 62).

Thrombocytopenia has been reported to be one of the symptoms of VCM-intoxication in man (24, 30, 46). Jühe et al. (24) suggested the use of periodic thrombocyte counts to be a diagnostic tool, because thrombocytopenia appeared to occur much earlier than any other sign of VCM-intoxication. This suggestion has, however, not been verified by the present observations in rats, because the number of thrombocytes was not found to be clearly influenced.

Failure of the blood to clot has been noticed in that guinea pigs died during exposure to an atmosphere containing 40 % VCM (42) and two workers of a PVC-factory from acute VCM-poisoning (11). In addition, Müller et al. (46) recently reported a disturbed thrombocytic function in 9 out of 17 patients suffering from "vinylchloride disease". However, rats exposed to 5 or 2 % VCM for 19 or 92 days respectively, had normal blood clotting times (32). In the present study prothrombin times were invariably shorter in VCM-exposed rats than in controls, though the differences were statistically significant only in males after 4 and 52 weeks. Because of the conflicting observations made in man and experimental animals, it is difficult to know whether any toxicological significance can be attached to the slight indication of hypercoagulability of the blood found in the experiment reported here. Nevertheless, in view of the need for a rapid diagnostic tool, it seems desirable to pay special attention to the possible effects of VCM on thrombocytes in future studies.

The chemical and biochemical examinations of the blood did not produce evidence of VCM affecting a certain parameter in particular.

The increases in blood urea nitrogen and the decreases in albumin and γ-globulins were only slight and occurred nearly exclusively after a test period of 52 weeks when many VCM-exposed animals had already died or were bearing tumours. In this respect it may be mentioned that in workers exposed to VCM Kramer and Mutchler (28) have found a positive correlation between the β-globulin content of the blood and the "time-weighted average exposure" (TWAE), whereas the haemoglobin content appeared to be negatively correlated with "TWAE". Gamma glutamic transpeptidase (γ-GT) determinations have been reported to be the most useful laboratory test for detecting liver damage in VCM- and PVC-workers (33). In the present studies no γ-GT determinations were done, because in our experience (56) and according to the findings of other investigators (4) the activity of this enzyme in the blood serum of rats is hardly above the detection limit.

The results of the above mentioned study by Kramer and Mutchler (28) also showed retarded excretion of bromosulphthalein in workers exposed to relatively high levels of VCM (150-300 ppm) indicating reduced liver function. The opposite effect of VCM on bromosulphthalein excretion was noticed in the present study with rats from week 13. The increased excretion of bromosulphthalein was paralleled by increases in relative liver weight. The more rapid excretion of BSP is, therefore not necessarily indicative of an improved liver function, but may rather be ascribed to an increased amount of active liver tissue.

There were slight indications of increased potassium levels in the blood serum of the test animals. The increases were only slight and possibly irrelevant. On the other hand, in man elevated levels of potassium in the blood point to impaired function of the renal glomeruli and/or the adrenal cortex (21).

In the final stage of the experiment (week 52) VCM-exposed animals produced - in comparison with the controls - more urine, the specific gravity and uric acid content of which were significantly diminished. These findings may point to an effect of VCM on the kidneys. Observations indicative of VCM adversely affecting the kidneys were: increased blood urea nitrogen levels, elevated kidney weights and a clearly increased degree of progressive nephrosis. The data from the literature concerning an effect of VCM on the kidneys are conflicting. Histopathologic l

changes in the kidneys including hyperaemia, glomerular changes and degeneration of tubular epithelium have been reported to occur in rats and other animal species following exposure to VCM at high concentrations varying from 3 to 30 % (42, 49, 53, 67). Torkelson et al. (65) found interstitial and tubular changes in the kidneys of rats exposed to VCM at the relatively low level of 500 ppm, 7 hr/day, 5 days/week, for a period of 4.5 months. In sharp contrast to these observations are the results of Lester et al. (32), who did not show any morphological renal alterations in rats exposed to VCM at levels of 5 and 2 % (8 hr/day) for periods of 19 and 92 days respectively. Renal tumours (nephroblastomas) have been found in rats following repeated exposures to VCM at concentrations varying from 50 to 10000 ppm (40). In the present study renal tumours were not observed. However, one of the test animals killed after 52 weeks showed atypical proliferation of renal tubular epithelium, which might be considered an early sign of tumour development at a later stage.

The α-fetoprotein determinations did not give indications of the presence of tumours producing this fetoglobulin, despite the fact that some of the test animals examined after 52 weeks had either liver angiosarcoma or hepatocellular carcinoma. Similar negative results have been obtained during a systematic programme for the detection of liver damage or angiosarcoma in workers exposed to VCM or its polymers (33). In all 1183 employees examined, normal α-fetoprotein values were found, although two new cases of hepatic angiosarcoma were detected. Non-neoplastic hepatic diseases may be accompanied by elevated α-fetoprotein levels (6a, 29, 56a, 59a). In the present study, aspecific liver damage, such as necrosis, degeneration and foci of cellular alterations, occurred in nearly all rats killed after 52 weeks, and is, therefore, most probably responsible for the slight, though statistically significant increases in the mean α-fetoprotein values found in the test groups towards the end of the experimental period.

Slightly increased relative heart weights in the test group and focal degeneration of the myocard in several VCM-exposed animals killed after 52 weeks are findings suggestive of an adverse effect of VCM on the heart. To the best of our knowledge, such cardiac effects of VCM have not been reported before, although in this connection

it should be mentioned that cultured heart cells from chickens have been found to die within 24 hours after exposure to certain types of PVC (43). In the latter case, however, it was not clear whether VCM or another compound present in the plastic was responsible for the cytotoxic effect.

During the first half year of the experiment the respiratory tract was not visibly affected by VCM. Thereafter, haemorrhages and inflammatory changes in the lungs were observed in several rats bearing either brain tumours (primary or secondary tumours) or pulmonary metastases of liver tumours. In addition, primary tumours of the lungs and of the olfactory epithelium in the nasal cavity were found in several rats exposed to VCM. So far pulmonary tumours attributable to VCM have been reported to occur in rabbits (39), in mice (26, 36) and possibly in man (15, 45), but not in rats and hamsters. As far as we know, the induction of nasal cavity tumours by VCM has not been reported before. The majority of the tumours observed in the present experiment were classified as very malignant carcinomas originating from the olfactory epithelium and Bowman's glands. One tumour was called an esthesioneurocepithelioma and another one a carcinosarcoma. The morphological aspects of the tumours were comparable to those of the nasal cavity tumours found in hamsters and gerbils following treatment with carcinogenic nitrosamines (8, 52), though the impression existed that in general the VCM-induced neoplasms were less differentiated.

Both the gross and microscopic aspects of the morphology of the Zymbal gland tumours encountered in the present study were fully comparable to those of the ones found in this organ by Maltoni et al. (40) in their VCM-studies with rats. In contrast to these investigators, we did not observe pulmonary metastases.

Brain tumours that can be ascribed to VCM-exposure have been found in rats (36, 39, 40) and possibly in man (45). The results of our experiment support these findings in that one of the VCM-exposed rats had developed a tumour of the brain. However, the tumour observed was a malignant ependymoma, whereas those described by Maltoni et al. (39, 40) were neuroblastomas, although these authors gave a picture of an ependymoma in one of their publications (40).

Disappearance of granular and Purkinje cells, and degeneration of the cerebellum have been mentioned by Viola et al. (67) as most prominent parenchymal lesions occurring in rats exposed to 30000 ppm VCM. No such degenerative changes were seen in the present study.

The effects of VCM on the liver may be divided in effects on the parenchyma and effects on non-parenchymal tissues. Degenerative, hyperplastic and neoplastic alterations of the parenchyma have been reported to occur in several animal species and also in man, the evidence of hepatocellular tumours in persons exposed to VCM being anecdotal (5). The degenerative changes varied from fatty infiltration (42, 47), hydropic swelling with or without vacuolisation (32, 47) isolated-cell-necrosis (47, 53) and centrolobular vacuolisation (23) to severe chronic hepatitis (67). Hyperplasia was seen as "proliferation of hepatocytes" (5) and enlarged polymorphic parenchymal cells with big nuclei containing several nucleoli (35, 47). A few hepatomas have been found in rats exposed to VCM for a prolonged period of time (39, 40). In addition, Williamson (70) reported that preliminary histological examination of tumours found in the livers of rats following gastric intubation of VCM at levels of 300 and 30 mg/kg body weight suggested that some of these tumours were hepatocellular carcinomas. The results of the present study in rats were in full agreement with the observations mentioned above, and likewise, produced convincing evidence of VCM adversely affecting the hepatic parenchyma, the major alterations being swelling and malformations of mitochondria, increase in smooth endoplasmic reticulum, necrosis and polymorphism of hepatocytes, hyperplasia of hepatocytes and finally hepatocellular carcinomas.

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. Evaluating swelling of cells or cell organelles is often a precarious undertaking because inadequate fixation might be involved. However, in this case an artifact can practically be excluded, because (a) the swollen mitochondria were found after fixation by both immersion and perfusion, (b) swollen mitochondria were not observed in any of the controls, and (c) mitochondrial swelling has

also been found in liver cells of rats given VCM by the oral route (60). Moreover, swelling of mitochondria has been reported to occur in hepatocytes under various pathological conditions, such as dietary deficiency in riboflavin (63), hypoxia (27, 44), and exposure to CCl_4 or ethanol (12, 55).

In addition to swelling of mitochondria, prolonged exposure to 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 (7, 55). In this respect one might speculate on the oxygen required for the activation of the MFO-system being in competition with oxygen necessary for other cellular functions, e.g. the mitochondrial respiration. Since swelling of mitochondria is known to occur in hypoxic conditions, the swelling of mitochondria seen after exposure to VCM might be due to shortage of oxygen.

The reduction in glucose-6-phosphatase activity in "foci of cellular alterations" occurring in the livers of VCM-exposed rats is a finding comparable to the decreased activity of this enzyme seen by Scherer and Emmelot (57) in "islands" induced by diethylnitrosamine in the liver of rats. These investigators consider the "islands" to represent an obligatory stage in liver carcinogenesis.

The hepatic "foci of cellular alterations" also exhibited an increased alkaline phosphatase activity and a decreased acid phosphatase activity. The physiological or toxicological significance of these observations is not clear, and we are not aware of similar reactions found in "foci of cellular alterations" induced by carcinogens.

In addition to the parenchymal alterations, the livers of the test animals exhibited focal dilatation of sinusoids, focal proliferation of sinusoidal cells with or without atypia and multicentric angiosarcomas containing widely varying amounts of fibrous tissue. Although these impressive changes of the hepatic stroma are in accordance with the observations of other investigators in man and experimental animals exposed to VCM (5, 18, 35, 41, 47), it might be of interest to draw attention to certain differences between the previous and the present findings. Maltoni and Lefemine (38) have reported the occurrence of

hepatic and extrahepatic blood vessel ectasias and angiomas, and of angiosarcomas outside the liver in rats, mice and hamsters. None of these lesions were observed in the present experiment, with the possible exception of a primary angiosarcoma in the lungs.

In view of their morphology, the sinusoidal cell tumours were classified as angiosarcomas, and were thus considered to have developed from endothelial cells. In this respect an interesting finding was the absence or inactivity (as to acid phosphatase) of Kupffer-cells in areas where the sinusoids were distended, possibly indicating Kupffer-cells not to be involved in the neoplastic process.

Nodular subcapsular fibrosis, progressive portal fibrosis and focal increase of intralobular connective tissue fibres are some of the characteristics of VCM-induced hepatic injury in man (64), which have been suggested by Popper and Thomas (50) to precede angiosarcoma development ("fibrotic precursor stage"). In rats marked fibrosis has been seen only within angiosarcomas or as a reaction to extensive necrosis of liver parenchyma. Therefore, in contrast to observations in man, in rats a fibrotic precursor stage does not seem to exist.

From the present results it appeared that the hepatocytic changes were visible earlier than those of the sinusoidal lining cells. This might be indicative of the hepatic parenchyma being attacked earlier by VCM than the hepatic stroma. However, the relationship between the hepatocytic and sinusoidal cell alterations, if existing at all, is not clear.

Finally, an attempt was made to indicate the earliest VCM-related changes detected in the present study. Being aware of the fact that "a statistically significant difference between the control and test group" is not always synonymous with "a VCM-effect", the four following changes might represent early VCM-effects, i.e. growth retardation, shortened blood clotting time, increased potassium content of the blood serum and swollen and malformed mitochondria in hepatocytes. VCM-effects appearing at a later stage were: increased urea nitrogen content of the blood (not very convincing), enlargement of kidneys, liver and spleen, foci of cellular alterations in the liver, and an increased amount of smooth endoplasmic reticulum in liver cells. All other VCM-effects did not become visible until the final stage when tumours had already emerged in many VCM-exposed animals.

On account of the nature or in other cases the scantiness of the early VCM-effects observed in the present study, it seems unlikely that any of the parameters affected by VCM at an early stage are obviously suitable as tools for a timely diagnosis of "VCM-disease" in human beings. On the other hand, these and other observations made in the experiment reported here may indicate the direction in which such an "early diagnostic tool" should be looked for.

5. CONCLUSIONS

The study has led to the following conclusions:

a. VCM is a versatile carcinogen capable of inducing highly malignant tumours in various organs of rats.

b. The experiment did not produce parameters being at an early stage sufficiently and consistently influenced by VCM to render them obviously suitable as tools for early diagnosis of "VCM-disease" in human beings.

c. The effects of VCM on the parenchyma of the rat liver most probably precede those on the hepatic stroma.

d. The nasal cavity and the liver of rats are about equally important as target organs for the carcinogenic activity of VCM.

e. Tumours occurring in the brain of VCM-treated rats can only be classified as primary brain tumours if the possibility of metastasis from nasal cavity tumours can be definitely excluded.

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Table 1. Cumulative mortality[x]

group no.	VCM (ppm)	initial number of rats	total number of deaths at end of week						
			32	33	36	40	44	48	52[xx]
			males						
8114	0	62	0	0	0	0	0	0	1
8115	5000	62	0	1	1	3	11	20	23
			females						
8114	0	62	0	0	0	0	0	0	0
8115	5000	62	0	1	2	5	8	16	22

x After 4, 13, 26 and 52 weeks 10 rats/sex/group were killed. These are not included in the table.

xx In week 52 only 9 male and 10 female rats of the test group were still alive.

Table 2. Mean body weights

VCM (ppm)	body weight (g) and standard deviation (in brackets) at end of week								
	0	1	2	4	8	20	32	44	52
males									
0	87 (1)	129 (1)	169 (1)	246 (2)	329 (3)	441 (5)	489 (7)	524 (7)	523 (8)
5000	87 (1)	117^{xxx} (1)	151^{xxx} (2)	224^{xxx} (2)	298^{xxx} (3)	399^{xxx} (5)	432^{xxx} (7)	443^{xxx} (10)	441^{xxx} (11)
females									
0	77 (1)	103 (1)	123 (1)	157 (1)	192 (2)	240 (3)	257 (4)	288 (6)	291 (6)
5000	77 (1)	96^{xxx} (1)	116^{xxx} (1)	150^{xxx} (1)	184^{xx} (2)	228^{xx} (3)	247 (4)	263^{xx} (5)	257^{xx} (8)

^{xx} $p \leq 0.01$ ^{xxx} $p \leq 0.001$

Table 3. Mean haematological findings after 4, 13, 26 and 52 weeks

VCM (ppm)	haemo-globin (g/100 ml)	packed cell vol. (%)	erythro-cytes (10^6/mm^3)	throm-bocytes (10^3/mm^3)	prothrom-bin time (sec.)	leucocytes total (10^3/mm^3)	differential count (%) L	N	E	M
					males week 4					
0	15.1	45.8	7.0	775	39.0	14.1	92.2	7.5	0.3	0
5000	15.0	47.2^{x}	7.5^{xx}	797	35.7^{xx}	11.5	93.1	6.3	0.6	0
					females week 4					
0	15.0	46.0	7.2	875	34.4	10.9	92.3	7.0	0.7	0
5000	15.4	46.6	7.5	853	32.6	10.8	94.3	5.3	0.4	0
					males week 13					
0	15.4	46.7	8.6	651	37.8	14.1	83.5	15.5	1.0	0
5000	15.6	47.3	8.7	525	36.4	12.3	82.9	15.7	1.4	0
					females week 13					
0	15.7	47.6	8.1	561	34.4	10.3	81.9	17.0	1.1	0
5000	15.3	46.4	8.2	619	34.0	11.7	82.9	15.0	2.1	0
					males week 26					
0	16.6	48.8	8.5	829	36.9	10.3	90.9	8.2	0.9	0
5000	16.7	49.3	9.1^{x}	1026^{xx}	34.6	10.9	87.2	11.7	1.1	0
					females week 26					
0	16.2	46.5	8.0	922	32.4	8.6	93.1	6.4	0.5	0
5000	15.8	45.1	7.9	1015	30.5	9.3	85.2^{x}	13.7^{x}	0.9	0.2
					males week 52					
0	16.6	49 9	8.5	674	35.9	12.3	71.1	27.5	1.4	0
5000	14.8^{x}	44.7^{x}	7.8	740	31.3^{x}	18.9^{xxx}	71.4	26.4	2.1^{x}	0
					females week 52					
0	16.8	50.0	8.1	585	33.2	8.7	81.4	16.1	2.4	0.1
5000	15.5	45.6^{x}	7.5	467	30.9	13.3	71.1	27.3	1.6	0

^{x} $p < 0.05$ ^{xx} $p < 0.01$ ^{xxx} $p < 0.001$

Table 4. Mean biochemical blood values after 4, 13, 26 and 52 weeks

VCM (ppm)	sugar (mg %)	bun (mg %)	serum enzyme activities				serum proteins						serum electrolytes				
			GOT	GPT	AP	LDH	α-feto-protein (ng/ml)	TSP (g %)	albu-min (g %)	globulins (%) α	β	γ	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Cl (mg %)
males week 4																	
0	59	11	180	29	18.4	411	77	6.8	2.4	61	30	9	3478	274	101	25	347
5000	59	12	167	27	15.4^{x}	389	90	6.0^{xx}	2.4	58	32	9	3517	272	98^{x}	26	354
females week 4																	
0	62	13	161	23	12.8	348	96	5.9	2.3	57	30	13	3524	272	103	26	349
5000	62	16	158	22	13.7	294	88	6.1	2.2	59	30	11	3538	292^{x}	104	23^{xx}	356
males week 13																	
0	74	13	147	25	8.5	281	109	8.4	2.9	57	30	13	3534	280	111	25	339
5000	77	13	172	29	8.5	419^{xx}	126	8.7	2.9	54	31	15	3595	296	109	27	344
females week 13																	
0	77	16	171	27	5.8	276	69	7.3	3.3	50	26	24	3669	280	112	25	364
5000	72	17	169	29	5.2	331	79	7.1	3.2	50	28^{x}	22	3686	304^{x}	111	25	364
males week 26																	
0	74	12	125	36	10.3	211	65	8.0	2.6	55	29	17	3474	298	105	32	324
5000	68^{x}	13	144	36	10.2	152	91	8.8	2.7	56	28	16	3448	312	103	35	332
females week 26																	
0	73	14	185	66	11.7	243	102	7.8	3.0	49	30	22	3492	290	108	34	328
5000	74	18^{x}	193	53	11.2	213	147	8.5	3.0	52	28	20	3461	336	104	35	325
males week 52																	
0	81	15	116	24	8.2	322	31	8.8	2.8	55	32	13	3315	225	105	23	362
5000	78	24^{xx}	103	28	8.2	238	56^{x}	8.6	2.3^{xx}	56	34	10^{x}	3366	212	106	23	344
females week 52																	
0	74	15	107	40	8.0	170	34	8.1	3.3	45	37	19	3373	209	112	26	341
5000	74	18^{x}	99	38	9.1	111	77^{xx}	7.8	2.5^{xxx}	53	34	14^{x}	3431	199	111	24	362

^{x} $p < 0.05$ ^{xx} $p < 0.01$ ^{xxx} $p < 0.001$

Table 5. Results of urine analyses after 4, 13, 26 and 52 weeks

VCM (ppm)	appearance	pH	sugar	protein	occult blood	ketones	microscopic findings								
							RBC	WBC	epith.	amorph	cryst.	casts	bact.	WE	sperm
						males week 4									
0	yellow	6	–	+	–	–	–	–	+	+	++	–	+	–	+
5000	yellow	6	–	+	–	–	–	–	+	+	++	–	+	–	+
						females week 4									
0	yellow	6	–	+	–	–	–	–	+	+	+	–	+	–	
5000	yellow	6	–	+	–	–	–	–	+	+	+	–	+	–	
						males week 13									
0	yellow	6/7	–	++	–	–	–	–	±	+	++	–	+	–	+
5000	yellow	6	–	+++	–	–	–	–	±	+	+++	–	+	–	+
						females week 13									
0	yellow	6	–	++	–	–	–	–	+	+	++	–	+	–	
5000	yellow	6	–	+++	–	–	–	–	+	+	++	–	+	–	
						males week 26									
0	yellow	5	–	++++	–	–	–	–	±	±	+++	–	+	–	+
5000	yellow	5	–	++++	–	–	–	–	+	+	+++	–	+	–	+
						females week 26									
0	yellow	5	–	++++	–	–	–	–	±	+	++	–	+	–	
5000	yellow	5	–	++++	–	–	–	–	+	+	+++	–	+	–	
						males week 52									
0	dark yellow	6	–	++	–	–	–	–	+	+	++	–	+	–	+
5000	light yellow	6	–	++	–	–	–	–	±	±	+	–	+	–	+
						females week 52									
0	dark yellow	6	–	++	–	–	–	–	+	+	++	–	+	–	
5000	light yellow	6	–	++	–	–	–	±	+	+	++	–	+	–	

– = negative + = slight +++ = high RBL = red blood cells WBC = white blood cells
± = minimal ++ = moderate ++++ = very high WE = worm eggs

Table 6. Mean urinary findings after 4, 13, 26 and 52 weeks

VCM (ppm)	specific gravity	UGOT (R.F.-units)	volume (ml)	uric acid µg/ml	phenol red excretion in 1 hr (%)
		males week 4			
0	1.0308	19	6.5	791	36
5000	1.0326	17	5.9	894	36
		females week 4			
0	1.0326	20	4.6	597	69
5000	1.0343	20	4.3	637	67
		males week 13			
0	1.0560	14	4.0	862	31
5000	1.0573	14	3.2^{x}	1057	41^{xxx}
		females week 13			
0	1.0509	15	3.0	678	72
5000	1.0590	19	2.6	854^{x}	73
		males week 26			
0	1.0645	15	3.4	1101	40
5000	1.0602	20	3.8	1046	46
		females week 26			
0	1.0671	20	2.2	911	71
5000	1.0616	23	2.7	850	72
		males week 52			
0	1.0772	11	3.6	1052	53
5000	1.0602^{x}	18^{xx}	5.1^{xxx}	737^{xx}	50
		females week 52			
0	1.0609	12	2.5	861	60
5000	1.0541^{x}	23^{x}	3.7^{x}	729^{x}	63

^{x} $p < 0.05$ ^{xx} $p < 0.01$ ^{xxx} $p < 0.001$

Table 7. Results of liver function tests after 4, 13, 26 and 52 weeks

VCM (ppm)	males		females	
	BSP-extinction after 10 min. ($\times 10^{-3}$)	sleeping time (min.)	BSP-extinction after 10 min. ($\times 10^{-3}$)	sleeping time (min.)
		week 4		
0	64	54	50	64
5000	68	102^{xxx}	65^{xx}	77
		week 13		
0	342	102	282	163
5000	207^{xx}	99	192^{xx}	152
		week 26		
0	264	111	167	122
5000	203	115	101^{x}	99^{x}
		week 52		
0	412	115	327	102
5000	249	116	256	120

^{x} $P < 0.05$ ^{xx} $P < 0.01$ ^{xxx} $P < 0.001$

Table 8. Average body weights (in g) and average relative organ weights (in g/100 g body weight) and their standard deviations after 4, 13, 26 and 52 weeks in brackets.

VCH (ppm)	body weight	heart	kidneys	liver	spleen	brain	testicles/ ovaries	thymus	pituitary	thyroid	adrenals	lungs
males week 4												
0	271 (3)	.355 (.006)	.78 (.02)	4.73 (.05)	.202 (.005)	.67 (.01)	1.10 (.02)	.203 (.007)	.0032 (.0005)	.0069 (.0003)	.0146 (.0009)	-[1]
5000	244^{xxx} (7)	.349 (.006)	.80 (.02)	4.94 (.15)	.203 (.006)	.71 (.02)	1.16 (.03)	.227 (.013)	.0034 (.0003)	.0070 (.0009)	.0186^{x} (.0012)	-
females week 4												
0	176 (4)	.390 (.004)	.81 (.01)	4.26 (.10)	.212 (.006)	.92 (.02)	.0478 (.0050)	.260 (.018)	.0062 (.0005)	.0088 (.0010)	.0310 (.0019)	-
5000	154^{xxx} (4)	.391 (.009)	.85 (.02)	4.34 (.15)	.226 (.007)	.99^{x} (.03)	.0472 (.0049)	.296 (.015)	.0039^{x} (.0007)	.0079 (.0013)	.0325 (.0028)	-
males week 13												
0	385 (13)	.294 (.008)	.63 (.01)	3.41 (.12)	.148 (.004)	.48 (.01)	.86 (.02)	.117 (.006)	-	.0055 (.0009)	.0108 (.0013)	.50 (.03)
5000	344^{x} (12)	.317 (.010)	.69^{xx} (.01)	3.98^{xx} (.09)	.159 (.009)	.55^{x} (.02)	.92 (.03)	.0107 (.0009)	-	.0053 (.0006)	.0143 (.0015)	.49 (.02)
females week 13												
0	215 (6)	338 (.006)	.65 (.02)	2.93 (.08)	.179 (.008)	.79 (.02)	.0333 (.0036)	.140 (.013)	-	.0085 (.0016)	.0229 (.0007)	.54 (.01)
5000	202 (6)	359 (.006)	.74^{xx} (.02)	3.29 (.21)	.190 (.008)	.83 (.02)	.0346 (.0035)	.134 (.007)	-	.0097 (.0015)	.0262 (.0019)	.54 (.02)
males week 26												
0	470 (9)	.281 (.009)	.65 (.02)	3.44 (.09)	.130 (.007)	.43 (.01)	.79 (.03)	.052 (.006)	.0025 (.0004)	.0046 (.0005)	.0091 (.0005)	.41 (.02)
5000	428^{xxx} (11)	.292 (.008)	.65 (.01)	4.00 (.28)	.147 (.007)	.46 (.01)	.87^{x} (.02)	.052 (.004)	.0026 (.0003)	.0048 (.0004)	.0104 (.0006)	.44 (.01)
females week 26												
0	252 (6)	.363 (.008)	.65 (.01)	3.19 (.09)	.174 (.007)	.69 (.03)	.0285 (.0015)	.071 (.005)	.0050 (.0006)	.0061 (.0006)	.0196 (.0010)	[redacted]
5000	238 (5)	.398 (.017)	.74^{xx} (.02)	4.34^{xx} (.22)	.213^{xx} (.011)	.76 (.02)	.0340 (.0022)	.078 (.007)	.0056 (.0005)	.0080^{x} (.0006)	.0244^{xx} (.0011)	.56 (.02)
males week 52												
0	503 (11)	.276 (.009)	.60 (.01)	3.34 (.08)	.142 (.007)	.42 (.01)	.66 (.02)	-	.0027 (.0003)	.0059 (.0007)	.0101 (.0005)	.41 (.01)
5000	441^{xxx} (11)	.311^{xx} (.006)	.75^{xxx} (.02)	4.66^{xxx} (.16)	.223^{xx} (.022)	.46^{x} (.01)	.79^{xx} (.04)	-	.0024 (.0003)	.0084 (.0012)	.0118 (.0006)	.45 (.02)
females week 52												
0	286 (11)	367 (.016)	.63 (.02)	3.22 (.08)	.173 (.011)	.66 (.03)	.0426 (.0042)	-	.0040 (.0004)	.0071 (.0007)	.0184 (.0014)	.48 (.01)
5000	258 (9)	.404 (.017)	.82^{xxx} (.04)	5.63^{x} (1.00)	.264^{x} (.038)	.70 (.02)	.0433 (.0035)	-	.0053 (.0007)	.0083 (.0008)	.0224 (.0015)	.60^{x} (.04)

^{x} $P < 0.05$ ^{xx} $P < 0.01$ ^{xxx} $P < 0.001$

[1] not determined

Table 9. Site, type and incidence of histopathological changes observed in rats killed after 4 weeks[x]

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	10	10	10	10
LIVER				
1. foci of RES-cells occasionally accompanied by a few necrotic hepatocytes	5	5	3	2
2. small area of necrosis	1	0	0	0
3. periportal infiltration of round cells	1	3	0	2
4. minimal degree of bile duct proliferation	0	1	0	0
KIDNEYS				
1. minimal degree of tubular nephrosis	2	4	3	1
2. small infiltrates of round cells	1	0	1	0
3. slightly hyperplastic pelvic epithelium	1	0	0	0
4. unilateral hydronephrosis	1	2	0	1
LUNGS				
1. slight "cuffs" of lymphocytes	4	2	1	1
2. thickened arterial walls	1	1	0	0
3. focal accumulations of alveolar macrophages	1	0	0	0
4. focal thickening of interalveolar septa	1	1	0	0
5. focal hyperaemia	1	0	0	0

[x] Treatment-related changes were not observed.

Remark: Organs microscopically examined and not showing pathological changes at all are not mentioned in this table.

Table 10. Site, type and incidence of histopathological changes observed in rats killed after 13 weeks[x]

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	10	10	10	10
LIVER				
1. FOCI OF CELLULAR ALTERATION:				
a. CLEAR CELL FOCI				
1. ONE OR A FEW FOCI	0	1	0	0
2. region containing periportal hepatocytes with cytoplasmic vacuoles and eosinophilic inclusions[xx]	0	1	0	0
3. foci of RES-cells occasionally accompanied by a few necrotic hepatocytes	2	2	5	3
4. periportal infiltrates of round cells	0	0	2	0
KIDNEYS				
1. minimal degree of tubular nephrosis	4	5	0	1
2. small infiltrates of round cells	2	0	0	2
3. unilateral hydronephrosis	0	0	1	0
4. proteinaceous droplets in tubular epithelial cells	0	2	0	0
LUNGS				
1. "cuffs" of lymphocytes				
a. slight	4	5	4	6
b. moderate	3	3	1	1
2. focal accumulations of alveolar macrophages	0	1	0	0
3. focal pneumonitis	2	1	2	4

[x] Treatment-related changes are written in capitals.

[xx] Probably related to treatment

Remark: Organs microscopically examined and not showing pathological changes at all are not mentioned in this table.

Table 11. Site, type and incidence of histopathological changes observed in rats killed after 26 weeks[x]

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	10	10	10	10
LIVER				
1. FOCI OF CELLULAR ALTERATION				
a. CLEAR CELL FOCI				
1. ONE OR A FEW	0	5	0	6
2. SEVERAL	0	3	0	0
b. BASOPHILIC FOCI				
1. ONE OR A FEW	0	2	0	2
2. SEVERAL FOCI	0	1	0	0
2. a few isolated large hepatocytes with finely vacuolated or eosinophilic cytoplasm[xx]	0	1	0	0
3. foci of RES-cells occasionally accompanied by a few necrotic hepatocytes	3	2	2	3
4. minimal degree of bile duct proliferation	1	0	0	0
CERUMINOUS GLANDS				
1. SQUAMUS CELL CARCINOMA	0	0	0	1
KIDNEYS				
1. tubular nephrosis				
a. minimal	6	6	1	2
b. slight	1	0	0	0
2. proteinaceous droplets in tubular epithelial cells	1	0	0	0
3. brownish pigment granules in tubular epithelium	1	0	0	0
4. minimal degree of nephrocalcinosis	0	0	0	1
LUNGS				
1. "cuffs" of lymphocytes				
a. slight	0	1	0	0
b. moderate	0	0	0	1
2. focal accumulation of alveolar macrophages	1	1	0	0
NASAL CAVITY				
1. slight rhinitis	0	2	0	0

[x] Treatment-related changes are written in capitals

[xx] Probably related to treatment

Remark: Organs microscopically examined and not showing pathological changes at all are not mentioned in this table.

Table 12. Site, type and incidence of histopathological changes observed in rats killed after 52 weeks[x]

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	10	9	10	10
LIVER[xx]				
1. FOCI OF CELLULAR ALTERATION:				
a. CLEAR CELL FOCI:				
1. ONE OR A FEW	2	0	2	0
2. SEVERAL	0	2	0	4
3. MANY	0	5	0	1
b. BASOPHILIC FOCI:				
1. ONE OR A FEW	0	3	0	4
2. HYPERPLASTIC LIVER CELL NODULE WITH ATYPIA	0	0	0	1
3. WELL DIFFERENTIATED HEPATOCELLULAR CARCINOMA	0	1	0	1
4. PROLIFERATION OF ATYPICAL SINUSOIDAL CELLS ONLY	0	1	0	1
5. ANGIOSARCOMA[xxx]	0	3	0	6
6. EXTENSIVE AREAS OF NECROSIS + BLOOD-FILLED CYSTS	0	1	0	0
7. DISTENDED SINUSOIDS	0	3	0	4
8. foci of RES-cells occasionally accompanied by a few necrotic hepatocytes	5	0	4	2
9. periportal infiltrates of round cells	1	1	1	0
10. slight degree of bile duct proliferation	1	1	0	0
CERUMINOUS GLANDS (ZYMBAL GLAND)				
1. HYPERPLASIA ONLY	0	1	0	0
2. SQUAMOUS CELL CARCINOMA	0	3	0	2
3. PAPILLOMATOUS HYPERPLASIA AND INFLAMMATION	0	1	0	1
4. INFLAMMATION IN EAR DUCT REGION	0	1	0	0
LUNGS				
1. METASTASES OF LIVER ANGIOSARCOMAS	0	1	0	3
2. PAPILLARY ADENOMA	0	0	0	1
3. ADENOMATOID LESION	0	3	0	1
4. HAEMORRHAGES	0	2	0	1
5. FOCAL, INCREASED CELLULARITY OF INTERALVEOLAR SEPTA	0	3	0	2
6. "cuffs" of lymphocytes:				
a. slight	1	1	2	2
b. moderate	2	0	0	0
7. thickened arterial walls	0	0	0	1
8. focal accumulations of alveolar macrophages:				
a. slight	0	1	0	0
b. moderate	0	3	0	1

Table 12 (continued 1)

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
KIDNEYS				
1. TUBULAR NEPHROSIS:				
a. SLIGHT	6	1	2	3
b. MODERATE	2	3	0	5
c. SEVERE	1	1	0	0
d. VERY SEVERE	0	4	0	0
2. FOCAL ATYPICAL PROLIFERATION OF TUBULAR EPITHELIUM	0	1	0	0
3. slight focal hyperplasia of pelvic epithelium	0	0	1	0
SPLEEN				
1. INCREASED HEAMATOPOIETIC ACTIVITY:				
a. SLIGHTLY	0	4	3	1
b. MODERATELY	0	0	1	2
c. STRONGLY	0	2	0	4
2. focal fibrosis	1	0	0	0
3. much iron pigment	0	1	2	0
HEART				
1. FOCAL MYODEGENERATION	0	3	1	8
2. THICKENED WALLS OF ARTERIES	0	2	0	4
3. lymphocytic infiltrate	0	1	0	0
NASAL CAVITY				
1. HYPERPLASTIC OLFACTORY EPITHELIUM ONLY	0	3	0	0
2. CARCINOMA OF OLFACTORY EPITHELIUM	0	1	0	5
3. rhinitis accompanied by hyperplastic epithelium	2	2	0	1
4. increased number of mucous cells	1	0	0	0
STOMACH				
1. dilated mucosal glands	1	1	2	1

Table 12 (continued 2)

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
PANCREAS				
1. Focal transformation of acini into duct-like structures	1	1	0	0
2. focal fibrosis	1	2	0	1
3. lymphocytic infiltrate	2	2	1	1
4. foci of macrophages with Fe-pigment	2	1	0	0
5. focal peritonitis	0	1	0	0
THYROID				
1. activation:				
a. slight	6	1	6	6
b. moderate	3	5	0	1
c. severe	1	1	0	0
2. ultimo-branchial remnants	1	0	0	0
3. psammoma bodies	2	1	0	1
EXORBITAL LACHRYMAL GLANDS				
1. prosoplasia:				
a. slight	5	2	0	0
b. moderate	3	3	1	0
c. severe	1	1	0	0
2. lymphocytic infiltrate	1	2	0	0
ADRENALS				
1. diffuse vacuolisation in zona fasciculata	2	1	0	0
2. focal "fatty" vacuolisation in cortex	1	1	1	2
3. stagnant pool of blood	0	0	0	1
SUBLINGUAL SALIVARY GLANDS				
1. hyperplastic epithelium of excretory duct	2	0	0	1
2. focal proliferation of demilune cells	1	0	0	2
HARDERIAN GLANDS				
1. adenitis	3	2	2	2

Table 12 (continued 3)

site and type of lesions	incidence of lesions			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
COECUM				
1. parasite	1	0	0	1
EYES				
1. keratitis	0	1	0	0
PREPUTIAL GLANDS				
1. adenitis	1	3	0	3
TESTES				
1. unilateral atrophy	2	1		
2. some atrophic seminiferous tubules	0	1		
3. hyperplastic interstitial cells	0	1		
PROSTATE				
1. prostatitis	1	1		
2. granular colloid	5	6		
OVARIES				
1. cyst			0	1
UTERUS				
1. pyometra			0	1
2. polyp			0	1

x Treatment-related changes are written in capitals

xx One female rat had two different types of liver tumours (hepatocellular carcinoma and angiosarcoma).

xxx In 3 cases anaplastic carcinoma could not be excluded.

Remark: Organs microscopically examined and not showing pathological changes at all are not mentioned in this table.

Table 13. Site, type and incidence of tumours in two groups of rats, each initially consisting of 62 males and 62 females, exposed to 0 and 5000 ppm VCM respectively[x, xx]

site and type of tumours	incidence of tumours			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	62	62	62	61
LIVER[xxx]				
1. HYPERPLASTIC NODULE WITH ATYPIA	0	0	0	3
2. WELL DIFFERENTIATED HEPATOCELLULAR CARCINOMA	0	1	0	2
3. ANGIOSARCOMA[xxxx]	0	6	0	16
CERUMINOUS GLANDS				
1. PAPILLOMA	0	2	0	0
2. SQUAMOUS CELL CARCINOMA	0	5	0	3
3. ADENO-SQUAMOUS CARCINOMA	0	0	0	1
LUNGS				
1. PAPILLARY ADENOMA	0	0	0	1
2. MESENCHYMAL TYPE OF TUMOUR (ANGIOSARCOMA?)	0	0	0	1
3. METASTASIS OF ANGIOSARCOMA OR ANAPLASTIC CARCINOMA OF THE LIVER	0	2	0	9
BRAIN				
1. MALIGNANT EPENDYMOMA	0	1	0	0
2. METASTASIS OF NASAL CAVITY CARCINOMA[xxxx]	0	6	0	2
3. METASTASIS OF MESENCHYMAL TYPE OF LUNG TUMOUR	0	0	0	1
NASAL CAVITY				
1. CARCINOMA OF OLFACTORY EPITHELIUM	0	9	0	9
2. CARCINO-SARCOMA	0	0	0	1
3. ESTHESIONEUROEPITHELIOMA	0	1	0	0
KIDNEYS				
1. METASTASIS OF MESENCHYMAL TYPE OF LUNG TUMOUR	0	0	0	1

Table 13 (continued)

site and type of tumours	incidence of tumours			
	males		females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
OVARIES				
1. Granulosa cell tumour			1	0

x Ten rats/sex/group were killed after 4, 13, 26 and 52 weeks (only 9 males and 10 females of the test group were still alive in week 52). The remaining control animals were killed in week 53.

xx Treatment-related tumours are written in capitals.

xxx One female rat had two different types of liver tumours (hepatocellular carcinoma and angiosarcoma).

xxxx In five cases anaplastic carcinoma could not be excluded.

xxxxx In two cases the tumours might have been a primary brain tumour.