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ONE-YEAR TIME-SEQUENCE INHALATION TOXICITY STUDY OF VINYL CHLORIDE IN RATS. II. MORPHOLOGICAL CHANGES IN THE RESPIRATORY TRACT, CERUMINOUS GLANDS, BRAIN, KIDNEYS, HEART AND SPLEEN*

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SUMMARY

Wistar rats were exposed to atmospheres containing 0 (control) or 5000 ppm vinyl chloride monomer (VCM), 7 h/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 examinations. The present paper describes the morphological changes observed in the respiratory tract, ceruminous glands, brain, kidneys, heart and spleen.

The VCM-effects included increased degree of tubular nephrosis, mild focal degeneration of the myocard, and increased haematopoietic activity in the spleen. In addition, primary tumours were found in the brain (ependymoma), lungs (papillary adenoma and mesenchymal type of tumour), ceruminous glands (mainly keratinized squamous cell carcinomas), and nasal cavity (carcinomas of the olfactory epithelium, carcino-sarcoma, esthesioneuroepithelioma).

INTRODUCTION

Studies in several animal species have shown that exposure to vinyl

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Abbreviation: VCM, vinyl chloride monomer.

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chloride monomer (VCM) may lead to morphological changes including neoplasms in various organs [1,6—10,13,15,17,21,22,24,25].

A recent inhalation experiment with VCM was carried out using rats in which haematological, biochemical and histological observations were made at intervals over a 1-year period. This investigation was undertaken in an effort to detect the earliest alterations attributable to VCM and to study the sequence of events leading to the formation of tumours. The results are presented in 3 papers of which this is the second. It deals with the morphological changes in the respiratory tract, ceruminous glands, brain, kidneys, heart and spleen. The first paper describes the design of the study, growth, mortality and results of haematological and biochemical examinations [4]. The third paper deals with the pathological alterations found in the liver [5].

MATERIALS AND METHODS*

2 groups of Wistar rats, each consisting of 62 males and 62 females, were exposed to atmospheres containing 0 or 5000 ppm VCM, 7 h/day, 5 days/week, for a period of 52 weeks. After 4, 13, 26 and 52 weeks, 10 rats/sex/group were killed and subjected to a careful gross examination. Samples of a wide range of organs and tissues were preserved in 10% buffered formalin, processed through paraffin, sectioned at 5 μm, stained with haematoxylin and eosin, and examined microscopically.

Special stains were applied to selected sections of the nose and brain; these included periodic acid Schiff, Gomori's reticulin stain, phosphotungstic acid-haematoxylin, Masson's trichrome stain and the stain for neurofibrils according to Glees and Marsland.

RESULTS

Lesions found in animals killed after 4, 13 or 26 weeks

Treatment-related pathological changes in organs other than the liver** were not observed in animals killed after 4, 13 or 26 weeks, except for a wart-like growth 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 squamous cell carcinoma arising from the ceruminous gland (gland of Zymbal).

Lesions found in animals killed after 52 weeks

A variety of neoplastic and non-neoplastic alterations attributable to VCM was seen in rats killed after 52 weeks (Table I).

A remarkable finding was the occurrence of hyperplastic and neoplastic alterations of the olfactory epithelium in the nasal cavity of several test animals. The hyperplastic changes were seen as proliferations of atypical

* For details not presented here the reader is refered to a previous paper [4].

** The pathological changes found in the liver have been described in a separate publication [5].

TABLE I

TYPE AND INCIDENCE OBSERVED IN ORGANS 52 WEEKS

Site and type of tumours
Number of animals examined
Ceruminous glands (Zymbal)
(1) Hyperplasia
(2) Squamous cell carcino
(3) Papillomatous hyperp inflammation
Lungs
(1) Metastases of liver and
(2) Papillary adenoma
(3) Adenomatoid lesion
(4) Haemorrhages
(5) Focal, increased cellul interalveolar septa
Kidneys
(1) Progressive nephrosis
a. Slight
b. Moderate
c. Severe
d. Very severe
(2) Focal proliferation of tubular epithelium
Spleen
(1) Increased haematopoie
a. Slightly
b. Moderately
c. Strongly
Heart
(1) Focal myodegenerati.o
(2) Thickened walls of art
Nasal cavity
(1) Hyperplastic olfactory only
(2) Carcinoma of olfactory

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Total incidence and mor

Location, type and to
Table II.

TABLE I

TYPE AND INCIDENCE OF VCM-RELATED HISTOPATHOLOGICAL CHANGES OBSERVED IN ORGANS OTHER THAN THE LIVER OF RATS KILLED AFTER 52 WEEKS

Site and type of tumours	Incidence of lesions			
	Males		Females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Number of animals examined	10	9	10	10
Ceruminous glands (Zymbal glands)				
(1) Hyperplasia	0	1	0	0
(2) Squamous cell carcinoma	0	3	0	2
(3) Papillomatous hyperplasia and inflammation	0	1	0	1
Lungs				
(1) Metastases of liver angiosarcoma	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
Kidneys				
(1) Progressive 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 proliferation of atypical tubular epithelium	0	1	0	0
Spleen				
(1) Increased haematopoiesis				
a. Slightly	0	4	3	1
b. Moderately	0	0	1	2
c. Strongly	0	2	0	4
Heart				
(1) Focal myodegeneration	0	3	1	8
(2) Thickened walls of arteries	0	2	0	4
Nasal cavity				
(1) Hyperplastic olfactory epithelium only	0	3	0	0
(2) Carcinoma of olfactory epithelium	0	2	0	5

basal cells and cells of Bowman's glands. The tumours were highly infiltrative carcinomas.

Total incidence and morphology of tumours

Location, type and total number of tumours observed are presented in Table II.

TABLE II

SITE, TYPE AND INCIDENCE OF TUMOURS FOUND IN ORGANS OTHER THAN THE LIVER IN 2 GROUPS OF RATS, EACH INITIALLY CONSISTING OF 62 MALES AND 62 FEMALES, EXPOSED TO 0 OR 5000 ppm VCM RESPECTIVELY*

Site and type of tumours	Incidence of tumours			
	Males		Females	
	VCM (ppm)		VCM (ppm)	
	0	5000	0	5000
Ceruminous glands				
Papilloma	0	2	0	0
Squamous cell carcinoma	0	5	0	3
Adeno-squamous carcinoma	0	0	0	1
Lungs				
Papillary adenoma	0	0	0	1
Mesenchymal type of tumour (angiosarcoma?)	0	0	0	1
Brain				
Malignant ependymoma	0	1	0	0
Metastasis of nasal cavity carcinoma**	0	6	0	2
Metastasis of mesenchymal type of lung tumour	0	0	0	1
Nasal cavity				
Carcinoma of olfactory epithelium	0	9	0	9
Carcino-sarcoma	0	0	0	1
Esthesioneouroepithelioma	0	1	0	0
Kidneys				
Metastasis of mesenchymal type of lung tumour	0	0	0	1
Ovaries				
Granulosa cell tumour			1	0

*Lesions considered to be treatment-related are written in capitals. 10 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.
**In 2 cases the tumours might have been primary brain tumours.

Most of the neoplasms found in the ceruminous glands were grossly seen as wart-like tissue masses which, upon microscopy, appeared to be well-differentiated, highly keratinized squamous cell carcinomas. In 2 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 2 VCM-exposed rats. 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

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Fig. 1. Carcinoma
5000 ppm VCM fc

classified as a malignant type of tumour, possibly a poorly differentiated angiosarcoma.

A few animals of the test group had a deformed nose due to a small focal swelling suggestive of a nasal tumour. Microscopic examination revealed malignant tumours in the nose of not less than 20 VCM-exposed rats. 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 nasal bones over large areas and occasionally extended to organs outside the nose, e.g. the brain.

18 of the neoplasms were diagnosed as carcinomas originating in the olfactory epithelium (Figs. 1 and 2). They predominantly consisted of

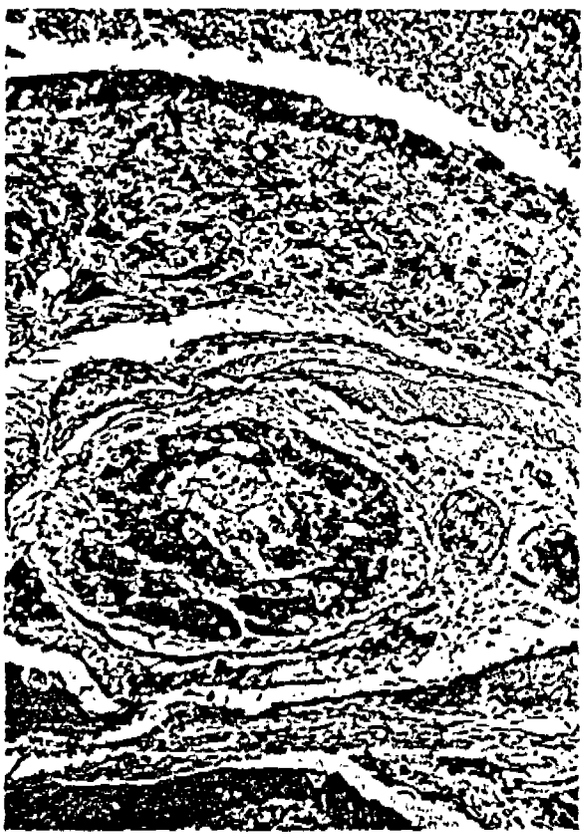

Fig. 1. Carcinoma of the olfactory epithelium in the nasal cavity. Male rat exposed to 5000 ppm VCM for 40 weeks. H & E, x160.

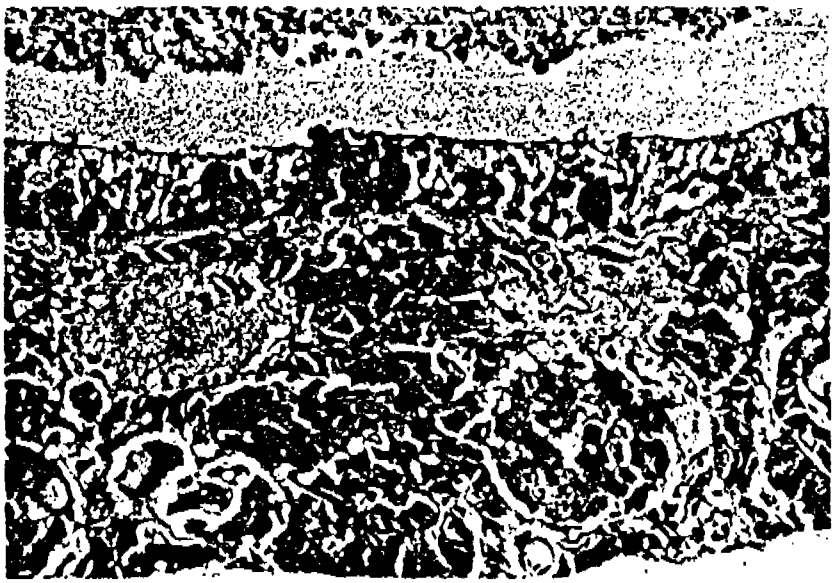

Fig. 2. Carcinoma of the olfactory epithelium in the nasal cavity. Note the fairly large vesicular nuclei with conspicuous nucleoli of the tumour cells. Male rat exposed to 5000 ppm VCM for 40 weeks, H & E, ×400.

Fig. 3. Esthesioneuroepithelioma in the nasal cavity. Note the columnar cells arranged in rosettes. Male rat exposed to 5000 ppm VCM for 40 weeks, H & E, ×160.

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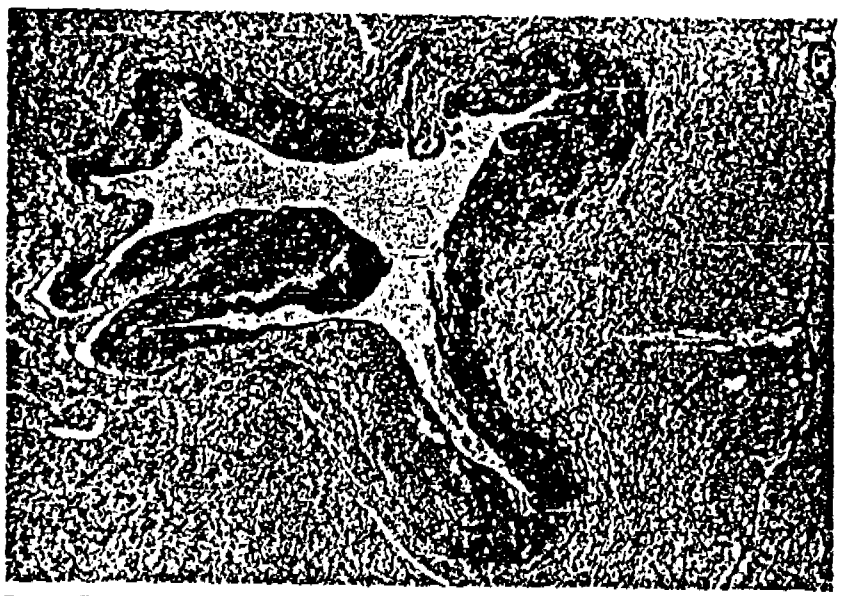

Fig. 4. Ependymoma in the anterior part of the cerebrum. The origin from the normal ependyma is clearly visible. Male rat exposed to 5000 ppm VCM for 44 weeks, H & E, ×40.

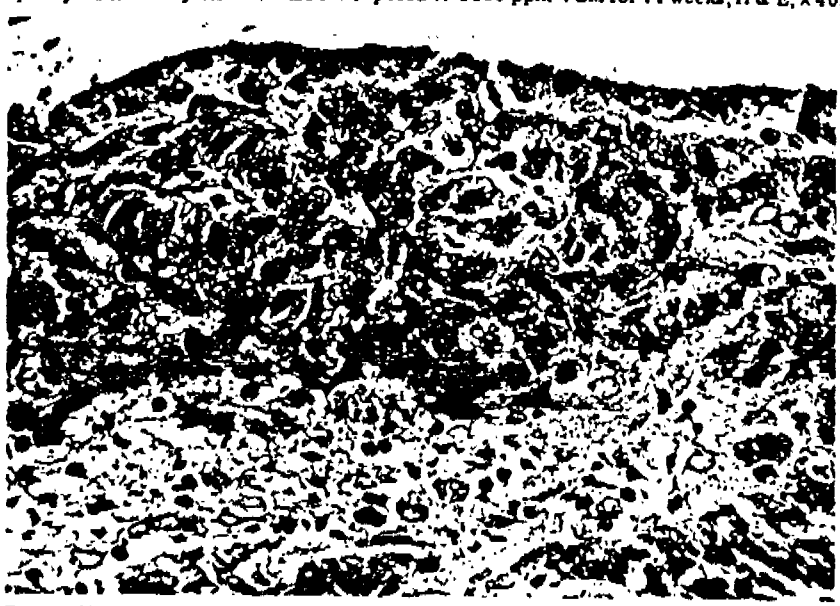

Fig. 5. Higher magnification of part of the ependymoma depicted in Fig. 4. Note the presence of rosette- or pseudorosette-like structures. The nuclei have a delicate, granular chromatin pattern; the nucleoli are inconspicuous. H & E, ×400.

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 1 of the nasal 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 (Fig. 3). The spaces lined by the tall cells often contained eosinophilic material. The histological

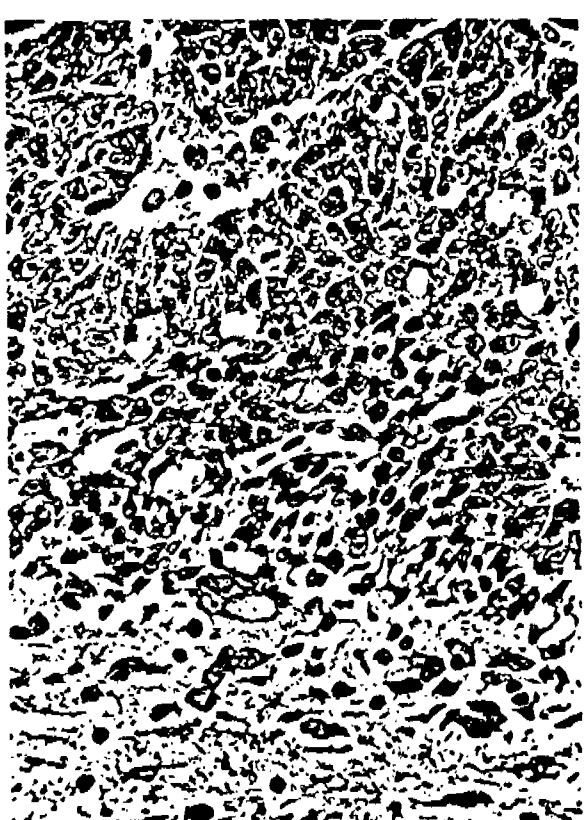

Fig. 6 Metastasis of nasal carcinoma in the brain. Note the close resemblance of the cells and in particular of the nuclei with those of the nasal tumour depicted in Fig. 2, and the striking difference in growth pattern and in appearance of cells and nuclei between this brain metastasis and the ependymoma depicted in Fig. 5. Female rat exposed to 5000 ppm VCM for 46 weeks. Reticulin stain, x 400.

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DISCUSSION

Several of the VCM-effects
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During the first half year of

appearance of the tumour was highly suggestive of esthesioneuroepithelioma, though a pure epithelial origin of the tumour could not be excluded.

Only 1 primary brain tumour was found. It occurred in the anterior part of the cerebrum of a male VCM-exposed animal killed in week 45. The tumour, which was not detected at gross examination, appeared to be in direct contact with 1 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 (Figs. 4 and 5).

Tumours found in 2 other test animals were located in the same part of the brain and morphologically resembled the above ependymoma although true rossettes were rare and a vascular reaction was absent. Since 1 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, 6 other VCM-exposed rats exhibited tumours of the anterior part of the brain which were easily recognized as metastases of nasal carcinomas (Fig. 6). Against this background, some hesitation might be justified as to the correctness of the above diagnosis of 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.

DISCUSSION

Several of the VCM-effects observed in the present experiment and described in this article, such as Zymbal gland tumours and an increased degree of tubular nephrosis were not unexpected and confirmed the results obtained by other investigators [12,16,18,20,23,24]. However, the occurrence of tumours in the nasal cavity came as a complete surprise.

Renal tumours (nephroblastomas) have been found in rats following repeated exposures to VCM at concentrations varying from 50 to 10 000 ppm [12]. In the present study renal tumours were not observed. However, 1 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.

Brain tumours that can be ascribed to VCM-exposure have been found in rats [11,12,14] and possibly in man [17]. The results of our experiment indicate the possibility that brain tumours actually are metastases rather than primary tumours. 1 of the VCM-exposed rats had developed a malignant ependymoma. In this respect it is interesting to note that the great majority of the cerebral tumours observed in VCM-exposed rats by Maltoni et al. [12] were neuroblastomas.

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 [11], in mice [8,9,14,22] and possibly in man [3,17] and rats [24]. 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. 1 tumour was diagnosed as esthesioneuroepithelioma and another as carcino-sarcoma. The morphological aspects of the tumours were similar to those of the nasal cavity tumours found in hamsters and gerbils following treatment with carcinogenic nitrosamines [2,19], though the impression existed that in general the VCM-induced neoplasms were less differentiated.

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REFERENCES

1 P.D. Berk, J.F. Martin, R.S. Young, J. Creech, I.J. Selikoff, H. Falk, P. Watanabe, H. Popper and L. Thomas, Ann. Int. Med., 84 (1976) 717.
2 A. Cardesa, P. Pour, H. Haas, J. Althoff and U. Mohr, Cancer, 37 (1976) 346.
3 H. Falk, and R.J. Maxweiler, Proc. R. Soc. Med., 69 (2976) 303.
4 V.J. Feron, A. Kruysse and H.P. Til, Toxicology, (1979).
5 V.J. Feron, B.J. Spit, H.R. Immel and R. Kroes Toxicology, (1979).
6 T.J. Haley, J. Toxicol. Environ. Health., 1 (1975) 47.
7 B. Holmberg, T. Kronevi and M. Winell, Acta Vet. Scand., 17 (1976) 328.
8 M.L. Keplinger, J.W. Goode, D.E. Cordon and J.C. Calandra, Ann. N.Y. Acad. Sci., 246 (1975) 219.
9 C.C. Lee, J.C. Bhandari, J.M. Winston, W.B. House, R.L. Dixon and J.S. Woods, J. Toxicol. Environ. Health, 4 (1978) 15.
10 D. Lester, L.A. Greenberg and W.A. Adams, Am. Ind. Hyg. Assoc. J., 24 (1963) 265.
11 C. Maltoni, G. Lefemine, P. Chieco and D. Carretti, Med. Lav., 65 (1974) 421.
12 C. Maltoni, G. Lefemine, P. Chieco and D. Carretti, Gli. Ospedali deila Vita, 1 (1974) 7.
13 C. Maltoni and G. Lefemine, Ann. N.Y. Acad. Sci., 246 (1975) 195.
14 C. Maltoni, Ambio, 4 (1975) 18.
15 C. Maltoni, Vinyl chloride carcinogenicity: an experimental model for carcinogenesis studies, in H.H. Hiatt, J.D. Watson and J.A. Winsten (Eds.), Origins of Human Cancer, Book A, Incidence of Cancer in Humans, Cold Spring Harbor Laboratory, 1977, p. 119.
16 E. Mastromatteo, A.M. Fischer, H. Christie and H. Danziger, Am. Ind. Hyg. Assoc. J., 21 (1960) 394.

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17 R.R. Monson, J.M. Peters and M.N. Johnson, Lancet, ii (1974) 397.
18 F.A. Patty, Industrial Hygiene and Toxicology, Vol. 2, Toxicology Interscience Publ., New York, 1963, pp. 1303.
19 P. Pour, A. Cartesa, J. Althoff and U. Mohr, Cancer Res., 34 (1974) 16.
20 L. Prodan, I. Suciu, V. Pîslaru, E. Ilea and L. Pascu, Ann. N.Y. Acad. Sci., 246 (1975) 159.
21 I.J. Selikoff and E.C. Hammond, Ann. New York Acad. Sci., 246 (1975) 1.
22 Y. Suzuki, Environ. Res., 16 (1978) 285.
23 T.R. Torkelson, F. Oyen and V.K. Rowe. Am. Ind. Hyg. Assoc., J., 22 (1961) 354.
24 P.L. Viola, A. Bigotti and A. Caputo, Cancer Res., 31 (1971) 516.
25 M. Winell. B. Holmberg and T. Kronevi, Environ. Health Persp., 17 (1976) 211.

ılk, P. Watanabe,

)76) 346.

) 328.
N.Y. Acad. Sci.,

nd J.S. Woods, J.

l., 24 (1963) 265.
.974) 421.
ella Vita, 1 (1974)

l for carcinogenesis
of Human Cancer,
Laboratory, 1977,

.nd. Hyg. Assoc. J.,