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# Experimental Research on Vinyl Chloride Carcinogenesis

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# INTRODUCTION

This report presents the conclusive results of the project of experimental carcinogenesis studies on vinyl chloride (VC) performed at Bentivoglio (BT) Laboratories of the Bologna Institute of Oncology.

The project was started in 1971, and lasted until 1983. It included more than 7,000 animals, the majority of which were submitted to chronic treatment. All were kept under observation until spontaneous death. More than 200,000 histological slides were examined. To our knowledge, these studies are the largest ever performed in cancerogenesis, in one laboratory, on a single compound.

This project produced a series of important information.

The project:

1) clearly demonstrated that VC is a carcinogen, which produces a variety of tumors in different tissues and organs;
2) indicated that liver angiosarcoma is the most specific (marker) VC-correlated tumor;
3) promoted the epidemiological investigations which led to the discovery of liver angiosarcoma in VC-exposed workers;
4) produced dose-response data, thereby providing the scientific bases for regulatory measures;
5) provided the scientific proofs that long-term carcinogenicity bioassays can predict oncogenic risk for humans, and be the basis for quantitative risk-assessment.

Together with the results of the BT Project, a review of the up-to-date available data on vinyl chloride carcinogenesis and correlated pathology is given.

The objective of the volume is to provide the Scientific Community and the Regulatory Agencies with the original results of our experiments. Therefore, numbers, tables, and graphs largely overcome words, comments, and discussions.

ers occupationally exposed to VC in several countries: USA (Ducatman et al., 1975; Heath et al., 1977); Sweden (Funes–Cravioto et al., 1975), UK (Purchase et al., 1978), Hungary (Szentesi et al., 1976) Norway (Hansteen et al., 1978), Belgium (Léonard et al., 1977), Czechoslovakia (Kucerová et al., 1979; Rössner et al., 1980).

IARC Monograph Supplementum (1982), on the basis of the data of Hansteen et al. (1978), Kucerová et al. (1979) and of Rössner et al. (1980) concluded "in follow-up studies, in which workers were exposed to levels that had been reduced to 15 ppm and lower, no aberration or sister chromatid exchange was reported. Sister chromatid exchange incidence dropped to a normal level shortly after termination of exposure to higher levels, however, the incidence of chromosomal aberrations returned to normal only after two years. (Thus, although sister chromatid exchanges were not observed in some studies, sampling may have occurred after the level returned to normal)."

## IV. CARCINOGENICITY

VC has been produced commercially since the end of the 20's. For more than forty years, neither experimental research, nor epidemiological and clinical investigations were undertaken to study and assess its possible carcinogenic potential.

### 1) Experimental Studies

In 1967, P. L. Viola, in an attempt to produce acro–osteolysis in animals, began the first long–term experiments in animals. A group of 26 male, 3–month–old Ar/IRE Wistar rats was exposed to an atmospheric concentration of 30,000 ppm commercial grade VC (99% pure) for 4 hours/day on 5 days/week for 12 months; the experiment was terminated at 54 weeks. Skin tumors developed in the submaxillary parotid region in all of the 17 surviving rats (14 epidermoid carcinomas, 2 mucoepidermoid carcinomas, 1 papilloma); in addition, lung tumors developed in 7 rats and osteochondroma in 5. No tumors were observed in 25 controls killed at unstated time. In his first report on the results of 12 months exposure, Viola (1970 a) described metaplastic changes in bones, which he considered to be similar to human acro–osteolysis. The data on cancerogenic effects of VC were disclosed for the first time by Viola (Viola, 1970 b) at the *10th International Cancer Congress* (Houston, 1970), and then extensively published in 1971 (Viola et al., 1971).

Immediately after the Houston report, we communicated with Professor Viola, who kindly put at our disposal the manuscript of his extensive report, then in press, plus several slides of the tumors. On the basis of his results and material, we reached the conclusion that the tumors described as cutaneous were carcinomas originating from Zymbal glands, and that the pulmonary tumors were most likely metastases from Zymbal gland carcinomas. The neoplastic lung lesions were in fact morphologically similar to Zymbal gland carcinomas, and all were observed in animals bearing Zymbal gland tumors.

On the basis of Viola's results, and those of early pioneer cytological sputum investigations, showing atypias in the respiratory epithelial cells (Maltoni et al., 1974 a), a systematic integrated plan of experiments was started at the Bentivoglio (BT) Research Laboratories of the Bologna Institute of Oncology.

The BT project began in 1971 and lasted for twelve years. It studied the carcinogenic effects of VC administered by different routes, at different dose-levels, and for different periods to animals of both sexes, of various species, strain, and age.

The results of these experiments indicated that:

- –VC was carcinogenic in all the tested animals, producing a variety of tumors, namely: in rats (Sprague–Dawley and Wistar): malignant mammary tumors (mainly carcinomas), Zymbal gland carcinomas, nephroblastomas, liver angiosarcomas, angiomas and fibroangiomas, extrahepatic angiosarcomas, angiomas and fibroangiomas, hepatomas, encephalic neuroblastomas, forestomach papillomas and acanthomas, and possible cutaneous epithelial tumors; in mice (Swiss): mammary carcinomas, liver angiosarcomas, angiomas and fibroangiomas, extrahepatic angiosarcomas, angiomas and fibroangiomas, lung adenomas (frequently with cellular atypias), and possibly hepatomas, forestomach papillomas and acanthomas and cutaneous epithelial tumors; in hamsters (Syrian Golden): liver angiosarcomas, forestomach papillomas, acoustic duct epithelial tumors, and possibly angiomas of other sites, cutaneous epithelial tumors, melanomas and hemolymphoreticular neoplasias;
- –VC was carcinogenic when administered by inhalation, ingestion, by prenatal exposure, and possibly by intraperitoneal and subcutaneous injection;
- –a correlation between concentration, daily dose, length of exposure, and tumors response was found;
- –newborn rats are more susceptible to the carcinogenic effects of VC on liver;
- –a variety of organs may be affected by the carcinogenic effects of VC in the same animal.

The results of the BT project have been reported in many congresses and have been published in numerous reports, since 1973. They are extensively reported in Part II of this volume, complete with the findings of the last experiments (BT4001, BT4006) recently finished, together with the complete references dealing with the BT project.

Viola and co–workers exposed, by inhalation, forty rabbits for 4 hours/day, 5 days/week for 12 months to 10,000 ppm VC. Between 9–15 months of exposure, 12 skin acanthomas and 6 lung adenocarcinomas were seen. No similar tumors occurred in 20 controls after 15 months of observation (Caputo et al., 1974).

Subsequent experiments, on a limited scale, confirmed the results of the BT project.

Groups of 100 male and 100 female CDI Swiss/ChR mice (age unspecified) were exposed to 2,500, 200, 50 ppm VC (purity unspecified) in air for 7 hours/day, 5 days/week for 9 months and were observed for an additional 9 months. Following 8 months' exposure, 49 treated animals died with tumors, meanwhile no tumors were observed in 200 controls (100 females, and 100 males). VC was found to produce lung adenomas, liver angiosarcomas, and mammary carcinomas. A dose–related carcinogenic effect was evident (Keplinger et al., 1975).

Two groups, each of 12 male and 12 female, 3-month-old NMRI outbred Albino mice, were exposed to 500 or 50 ppm VC in air for 6 hours/day, 5 days/week. The 500 ppm group was exposed for only 25 weeks (due to the poor condition of mice), the 50 ppm group was exposed for 52 weeks, at which

time the experiment was terminated. Groups of control animals were included in the experiments. Under these experimental conditions, VC caused, at high and low concentrations, pulmonary adenomas, extrahepatic angiosarcomas, and, at higher concentrations, an increase of mammary carcinomas, and one liver angiosarcoma (Holmberg et al., 1976).

Groups of 36 male and 36 female 2–month–old Albino CD mice were exposed to 1,000, 200, 50 and 0 ppm VC (99.8% pure) in air for 6 hours/day, 5 days/week for 52 weeks (end of the experiment); at that time, 70, 52, 46, and 38 animals respectively were still alive. VC was found to cause lung adenomas, liver angiosarcomas, extrahepatic angiosarcomas, and mammary carcinomas. A dose–related carcinogenic effect was evident for lung adenomas and liver angiosarcomas (Lee et al., 1977, 1978).

Groups of 36 male and 36 female 2–month–old CD rats were exposed to 1,000, 250, 50 and 0 ppm VC in air (99.8% pure) for 6 hours/day, 5 days/week for 52 weeks, at which time the surviving animals (72, 70, 58, and 51 respectively) were killed. Liver and lung angiosarcomas occurred in rats treated with 1,000 and 250 ppm. A dose–related carcinogenic effect was evident (Lee et al., 1977).

Groups of 62 male and 62 female Wistar rats were exposed to 5,000 and 0 ppm VC in air, 7 hours/day, 5 days/week for 52 weeks. Tumors were observed in liver (hepatocellular carcinoma and angiosarcoma), brain, ceruminous glands, and nasal cavities (Feron et al., 1979 b; Feron and Kroes, 1979).

Small groups of male, 5–week–old, CD1 Swiss/ChR mice were exposed to 6,000, 2,500 and 0 ppm VC in air for 5 hours/day, 5 days/week for 5 and 6 months. Under these experimental conditions the animals were sacrificed between 2 to 37 days following the end of the treatment. VC was found to cause lung adenomas in 26 of the 27 treated animals. None of these tumors were found in the 16 controls. Light and electron microscopic studies suggested that neoplastic cells originate from type II alveolar epithelium via its hyperplastic form (Suzuki, 1978, 1981).

Hehir et al. (1981, and Unpublished Report with limited circulation) studied the effects of the exposure to several concentrations (50,000, 5,000, 500, and 50 ppm) of VC vapor, administered 1 hour, once, on Fischer 344 rats, and in ICR mice. The same total dose, 5,000 ppm, was delivered in 10 (× 500 ppm) or 100 (× 50 ppm) times, for 1 hour/day, 5 days per week, to Fischer 344 rats, and to A/J mice. Animals of both sexes were used. The age of the animals at start varied from 8 to 21 weeks. The animals were kept under observation up to 24 months (rats), and up to 20 months (mice). Interim sacrifices were performed at different periods of the experimental biophase. The plan of the experiment and the most important findings are shown in Table 7. This study was sponsored by the Consumer Products Safety Commission (CPSC) (USA).

The most important results of this study are:

- –the enhancement of total tumors in Fischer rats, following 10 and 100 1-hour exposures to 500 and 50 ppm respectively;
- –the clear–cut enhancement of pulmonary tumors and of total tumors in ICR mice, following the exposure to 50,000 ppm for 1 hour;
- –the enhancement of total tumors and of pulmonary tumors (also of adenocarcinomas) in A/J mice, following 10 1-hour exposures to 500 ppm, and of total tumors, following 100 1-hour exposures to 50 ppm.

**TABLE 7**

**Plan and results of the experiments of Hehir et al. (1981).**

| Species and strain | Concentration (ppm) | Frequency of treatments | Sex | Group size | Lung tumors | | Total tumors |
|---|---|---|---|---|---|---|---|
| | | | | | Adenomas | Carcinomas | |
| Fischer 344 rat | 50,000 | 1 | M | 90 | | | 129 |
| | | | F | 88 | | | 107 |
| | | | M&F | 178 | | | 236 |
| | 5,000 | 1 | M | 85 | | | 135 |
| | | | F | 95 | | | 101 |
| | | | M&F | 180 | | | 236 |
| | 500 | 1 | M | 90 | | | 170 |
| | | | F | 100 | | | 121 |
| | | | M&F | 190 | | | 291 |
| | 50 | 1 | M | 90 | | | 178 |
| | | | F | 90 | | | 72 |
| | | | M&F | 180 | | | 250 |
| | 0 | — | M | 92 | | | 186 |
| | | | F | 79 | | | 89 |
| | | | M&F | 171 | | | 275 |
| Fischer 344 rat | 500 | 10 | M | 90 | | | 114 |
| | | | F | 90 | | | 108 |
| | | | M&F | 180 | | | 222 |
| | 0 | — | M | 50 | | | 105 |
| | | | F | 50 | | | 61 |
| | | | M&F | 100 | | | 166 |
| | 50 | 100 | M | 90 | | | 176 |
| | | | F | 90 | | | 110 |
| | | | M&F | 180 | | | 286 |
| | 0 | — | M | 50 | | | 96 |
| | | | F | 50 | | | 59 |
| | | | M&F | 100 | | | 155 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | F | 90 | | 110 |
| | | | M&F | 180 | | 286 |
| 0 | — | | M | 50 | | 96 |
| | | | F | 50 | | 59 |
| | | | M&F | 100 | | 155 |

**TABLE 7 (Continued)**
**Plan and results of the experiments of Hehir et al. (1981).**

| Species and strain | Concentration (ppm) | Frequency of treatments | Sex | Group size | Lung tumors[1] Adenomas | Lung tumors[1] Carcinomas | Total tumors |
|---|---|---|---|---|---|---|---|
| ICR mouse | 50,000 | 1 | M | 90 | 31/ 61 (51%) | 1/ 61 ( 2%) | 49 |
| | | | F | 90 | 14/ 76 (18%) | 2/ 76 ( 3%) | 61 |
| | | | M&F | 180 | 45/137 (33%) | 3/137 ( 2%) | 110 |
| | 5,000 | 1 | M | 90 | 14/ 65 (22%) | 1/ 65 ( 2%) | 33 |
| | | | F | 90 | 10/ 78 (13%) | 0/ 78 ( — ) | 45 |
| | | | M&F | 180 | 24/143 (17%) | 1/143 ( 1%) | 78 |
| | 500 | 1 | M | 90 | 8/ 66 (12%) | 0/ 66 ( — ) | 31 |
| | | | F | 90 | 10/ 73 (14%) | 1/ 73 ( 1%) | 51 |
| | | | M&F | 180 | 18/139 (13%) | 1/139 ( 1%) | 82 |
| | 50 | 1 | M | 90 | 8/ 71 (11%) | 0/ 71 ( — ) | 15 |
| | | | F | 90 | 6/ 68 ( 9%) | 0/ 68 ( — ) | 47 |
| | | | M&F | 180 | 14/139 (10%) | 0/139 ( — ) | 62 |
| | 0 | — | M | 82 | 4/ 50 ( 8%) | 0/ 50 ( — ) | 18 |
| | | | F | 88 | 8/ 70 (11%) | 0/ 70 ( — ) | 54 |
| | | | M&F | 170 | 12/120 (10%) | 0/120 ( — ) | 72 |
| A/J mouse | 500 | 10 | M | 90 | 56/76 (74%) | 12/ 76 (16%) | 87 |
| | | | F | 90 | 68/ 90 (76%) | 10/ 90 (11%) | 85 |
| | | | M&F | 180 | 124/166 (75%) | 22/166 (13%) | 172 |
| | 0 | — | M | 50 | 15/ 43 (35%) | 0/ 43 ( — ) | 20 |
| | | | F | 50 | 16/ 47 (34%) | 3/ 47 ( 6%) | 25 |
| | | | M&F | 100 | 31/ 90 (34%) | 3/ 90 ( 3%) | 45 |
| | 50 | 100 | M | 90 | 27/ 77 (35%) | 3/ 77 ( 4%) | 63 |
| | | | F | 90 | 38/ 81 (47%) | 4/ 81 ( 5%) | 74 |
| | | | M&F | 180 | 65/158 (41%) | 7/158 ( 4%) | 137 |
| | 0 | — | M | 40 | 11/ 39 (28%) | 2/ 39 ( 5%) | 21 |
| | | | F | 50 | 18/ 45 (40%) | 0/ 45 ( — ) | 20 |
| | | | M&F | 90 | 29/ 84 (35%) | 2/84 ( 2%) | 41 |

[1]Incidence of response/number evaluated.

Groups of male and female Sprague–Dawley rats of different ages (6,18,32 and 52 weeks) were exposed to 940 ppm VC by inhalation, 7 hours/day, 5 days/week for 24 weeks. In each age group there were 110 to 128 males, and the same number of females. Control groups, which were not exposed to VC, consisted of the same number of males and females, in each age group (Groth et al., 1981). All animals died spontaneously, were sacrificed moribund, or were killed at schedule times (3, 6 and 9 months after initial exposure). The authors summarized their results as follows: "The older the rats were when they were first exposed, the greater the incidence of angiosarcomas. The incidence of angiosarcomas in the four age groups (from youngest to oldest) in the exposed males, in the non–scheduled sacrifice groups were: 0/37 (0%); 0/44 (0%); 3/45 (6.7%); and 13/55 (24%). Similarly, for the females, these incidences were: 2/38 (5.3%); 7/47 (15%); 23/49 (47%); and 11/54 (20%). Most of the angiosarcomas were highly anaplastic, primary tumors in the livers that metastasized to the lungs. Only one angiosarcoma was seen in all the control rats; that occurred in subcutaneous tissues. This study demonstrated that older adult animals (in the studied range of ages) and females are more susceptible to the angiosarcoma–inducing effects of vinyl chloride than young adult animals and males, respectively."

Four treatment groups (80 male Sprague–Dawley rats per group) were treated as follows: inhalation of 600 ppm VC, 4 hours/day, 5 days/week for 52 weeks; VC, with the same regimen, and 5% ethanol in drinking–water (V/V) (the ingestion of ethanol was begun four weeks prior to inhalation of VC, and continued for life or termination of the study); ethanol, with the same regimen, and filtered air; and filtered air. The study lasted two and one–half years from the first VC exposure. Inhalation of VC induced liver angiosarcomas in 23% of the exposed animals; ethanol in addition to VC inhalation increased the incidence to 50%; concomitant administration of VC and ethanol also produced an excess of hepatocellular carcinomas and lymphosarcomas. Ethanol, with or without VC, had a strong tumorigenic effect on the endocrine system. In this model system, ethanol appears to potentiate the carcinogenic response to VC (Radike et al., 1981).

There are several carcinogenicity studies on PVC and on vinyl chloride–vinyl acetate copolymers, following subcutaneous or intraperitoneal implantation.

Squares or discs of a commercial PVC (containing some additives), 0.04 mm thick and 15 mm wide, were implanted into the subcutaneous tissue of the abdominal wall of 44 adult Wistar rats. Seventeen (38.6%) sarcomas (16 fibrosarcomas and 1 liposarcoma) developed at the site of implantation, with a latent period of 27-104 weeks. Similar perforated film did not produce local sarcomas in 27 treated rats. With a pure PVC film, 0.03 mm thick, in the same experimental conditions, 4 sarcomas at the site of implantation were obtained in a similar group of treated rats, after 76 weeks (Oppenheimer et al., 1952, 1955).

PVC film, of 4×5×0.16 mm, was implanted into the subcutaneous tissue of the abdominal wall of 35 adult, male and female, Wistar rats. A group of 25 rats received an implant of glass of similar size (control group). The experiment lasted 114 weeks. One sarcoma and 1 fibroma were observed at the site of the implant, after 83 weeks in PVC treated animals. No local tumors were observed in the control group (Russell et al., 1959).

PVC films (of unspecified size) were implanted by laparotomy to surround the kidney, in 80 outbred albino rats. 6/16 rats, that survived 41-53 weeks developed fibrosarcomas at the site of implantation (Raikhlin and Kogan, 1961).

PVC capsules, whole PVC films, or perforated PVC films, implanted in the kidney, in rats, caused sarcomas respectively in 5/16, 2/5 and 1/5 treated animals (Kogan and Tugarinova, 1959).

Pieces of vinyl chloride–vinyl acetate copolymer, of 15 × 22 × 0.2 mm, were implanted subcutaneously, in 82 male and female 6-8-week-old CBA/H-T6 mice. Sarcomas at the site of implantation developed in 65% of the males and in almost all of the females, after 30-52 weeks. No subcutaneous tumors were observed in a group of 80 untreated mice. Powder (particle size: 50-100 $\mu$) of the same material, corresponding by weight to two films of 15 × 22 × 0.2 mm, was implanted subcutaneously, in 30 male and 46 female 6-week-old, CBA mice. The animals were kept under observation until death. No treatment related tumors were observed. One sarcoma found in a female was attributed to clumping of the powder (Brand et al., 1967 a, b, 1975).

Films of vinyl chloride–vinyl acetate copolymer, of 15 × 22 × 0.2 mm and of 7 × 15 × 0.2 mm, were implanted into the subcutaneous tissue, in groups of 9-124 male and female mice of 18 strains, to test strain differences in response. The incidence of sarcomas at the site of the implants varied from strain to strain, and males appeared to be more responsive than females (except for AKR mice) (Brand et al., 1977).

**2) Human Data (case-reports and epidemiological studies)**

*A) Liver angiosarcoma*

Following the early 1973 results of the experiment of the BT project (Maltoni, 1974 a; Maltoni and Lefemine, 1974 a, b) in 1974, more than forty years after the introduction of VC in industry, Creech and Johnson (1974) reported for the first time an association of exposure to VC with cancer in man. Three cases of liver angiosarcoma were reported in men who were employed in VC–PVC industry, in a single VC polymerization plant in the USA. Further retrospective investigations showed that in previous years (precisely since 1955), workers exposed to VC had died of liver angiosarcoma (Stafford, 1983), but, in absence of experimental indications, these cases were misdiagnosed and not correlated with VC exposure.

In the same and the immediately following years (1975-1978), by reviewing medical records and pathological material, and by systematic medical screening, other liver angiosarcomas in workers exposed to VC, were reported in the USA, as well as in other countries: USA (Block, 1974; Falk et al., 1974; Makk et al., 1974); Canada (Noria et al., 1976; Makk et al., 1976; Delorme and Theriault, 1978); Romania (Lloyd, 1975); Czechoslovakia (Lloyd, 1975); Yugoslavia (Saric et al., 1976); Sweden (Byrén and Holmberg, 1975); Norway (Lloyd, 1975); Federal Republic of Germany (Lange et al., 1974, 1975); France (Ravier et al., 1975; Couderc et al., 1976; Roche et al., 1978); Italy (Maltoni, 1974 g); UK (Lee and Harry, 1974; Smith et al., 1976).

Up to 1983, 102 cases of liver angiosarcomas were listed in the VC Liver Angiosarcoma (ASL) Case Register (Stafford, 1983). The distribution of the

cases in the world, the average latency periods (from the first exposure, to diagnosis) and the incidence per annum are shown in Table 8 and in Table 9.

The incidence per annum shows a major concentration in the years 1972–1980.

In the ASL Case Register (Stafford, 1983) the list of 102 cases, the Italian case 01 (Maltoni, 1974 g; Maltoni and Rondinella, 1980) was removed, because it was estimated to be "not homogeneous". This case was observed in a PVC extruder of a small bag factory (PANSAC, near Venice, Italy): the latency period was rather short (6 years), the exposure had been limited to 3 years, and the angiosarcoma was observed, other than in the liver, also within the lungs and pericardium (the pulmonary and pericardic localization most probably were metastases, but a multicentric origin could not be excluded).

In April 1983, just at the time in which the ASL Case Register was printed, a second case of liver angiosarcoma was observed in a PVC extruder of the same PANSAC factory. The history of this tumor was much more homogeneous with the other cases reported in ASL Case Register (Stafford, 1983). The observation of 2 cases among PVC extruders of the same small factory leaves little doubt that the risk of developing liver angiosarcomas must be taken in consideration also for extruders (Maltoni et al., 1984 a).

This conclusion is important, mainly for two reasons: 1) the large numbers of PVC extruders through the world; 2) the fact that, up to present, the past concentrations of VC in PVC extrusion work-places were thought to be "safe" (a sort of safeguard level) (Maltoni et al., 1984 a).

**TABLE 8[1]**

**Distribution and latency period of VC occupational liver angiosarcomas (ASL).**

| Part of the World | Country | No. of Cases | Total Years | Average Latency Time (in years) |
|---|---|---|---|---|
| Western Europe | Belgium | 1 | 18 | 18 |
| | France | 14 | 336 | 24[3] |
| | Germany | 21 | 385 | 18.3[3] |
| | Italy | 3[2] | 58 | 19.3 |
| | Norway | 1 | 22 | 22 |
| | Sweden | 5 | 122 | 24.5 |
| | U.K. | 8 | 189 | 23.62 |
| | Total | 53 | 1130 | 21.32 |
| North America | U.S. | 31 | 769 | 24.80[3] |
| | Canada | 10 | 204 | 20.4 |
| | Total | 41 | 973 | 23.73 |
| Rest of World | Czechoslovakia | 2 | 30 | 15.0 |
| | Japan | 2 | 39 | 19.5 |
| | Yugoslavia | 4 | 85 | 21.2 |
| | Total | 8 | 154 | 19.2 |
| Summary | W. Europe | 53 | 1130 | 21.32 |
| | N. America | 41 | 973 | 23.73 |
| | Rest of World | 8 | 154 | 19.2 |
| | Total | 102 | 2257 | 22.12 |

[1]From Stafford, VC Occupational Angiosarcoma of Liver (ASL) Register (1983).
[2]Does not include Italian 01.
[3]Of the countries with <10 cases, France and USA have average latency period above world average and Germany well below.

TABLE 9[1]

Incidence of VC occupational liver angiosarcomas (ASL), by date of death.

| Year of death | No. |
|---|---|
| 1955 | 1 |
| 1956 | 0 |
| 1957 | 1 |
| 1958 | 0 |
| 1959 | 0 |
| 1960 | 0 |
| 1961 | 1 |
| 1962 | 1 |
| 1963 | 0 |
| 1964 | 1 |
| 1965 | 0 |
| 1966 | 1 |
| 1967 | 1 |
| 1968 | 5 |
| 1969 | 3 |
| 1970 | 2 |
| 1971 | 3 |
| 1972 | 5 |
| 1973 | 7 |
| 1974 | 6 |
| 1975 | 11 |
| 1976 | 11 |
| 1977 | 9 |
| 1978 | 10 |
| 1979 | 5 |
| 1980 | 10 |
| 1981 | 4 |
| 1982 | 1 |
| 1983 | 1 |
| Total | 100[2] |

[1]From Stafford, VC Occupational Angiosarcoma of Liver (ASL) Register (1983).
[2]This list does not include 2 U.S.A. cases, who are alive.

The pathology and the pathogenesis of liver angiosarcoma in VC–PVC workers, and the other more or less associated hepatic lesions, were extensively studied by Popper and Thomas (Popper and Thomas, 1975; Thomas and Popper, 1975).

Their observations and conclusions are reported in Table 10.

The stimulation and proliferation of hepatocytes have been considered important changes in relation to the pathogenesis of liver angiosarcoma. The activated hepatocytes, in fact, may be more active metabolically in the transformation of VC in active metabolite(s), which in turn affect(s) sinusoidal lining cells. This hypothesis is consistent with the observation of a topographical correspondence between activated hepatocytes and proliferating and dysplastic sinusoidal cells.

Examples of various patterns of liver angiosarcomas, observed in VC–PVC workers, are shown in Figures 1–4.

The changes observed in the livers of VC–PVC workers, are largely reproduced in experimental animals exposed to the monomer, in which, under particular experimental conditions, activated and proliferating hepatocytes progress to hepatocarcinoma (see, Part II of this volume).

**TABLE 10**

**Characterization and pathogenesis of hepatic lesions in VC-PVC workers, with particular regard to liver angiosarcoma.**

1) Sinusoidal dilatation and proliferation of sinusoidal cells. → Piling up and atypias of sinusoidal lining cells → **Angiosarcoma:**

**A) Histotype:**
- –infiltrative tumor
  - sinusoidal pattern
  - papillary pattern
  - cavernous pattern
- –nodular tumor
  - capillary pattern
  - anaplastic pattern

**B) Extension:**
- –multicentricity
- –infiltration (the tumor envelops liver cells and bile ductuli)

**C) Secondary changes in angiosarcoma**
- –hemorrhage
- –necrosis
- –marked fibrosis
- –pseudo-capsule

2) Stimulation of hepatocytes
- "megalocytosis"
- "little cell-big cell" changes

→ Proliferation of hepatocytes → **Regenerating nodules (?)** / **Hepatoma (?)**

3) Proliferation of bile ducts

4) Fibrosis capsule
- –portal tract
- –intralobular (perisinusoidal)

→ Portal hypertension → Splenomegaly, varices

Liver angiosarcoma and acro–osteolysis have been found not to be associated (Heath et al., 1975).

*B) Other tumors*

An excess of cancers, involving different organs and of different histotypes (other than liver angiosarcoma) has been correlated to VC occupational exposure, starting from 1974 (Wagoner, 1974).

The sites and types of tumors and the available evidence are exposed in Table 11

The most evident correlation is found between VC exposure and brain tumors. A picture of glioblastoma multiforme (astrocytoma–grade IV) in a worker of VC–PVC industry is shown in Figures 5 and 6.

Few isolated cases of hepatocarcinomas have been observed among workers of VC–PVC industries (Delorme et al., personal communication).

There have been rumors of an excess of digestive tract, urinary tract, and mammary carcinomas (in women) among people occupationally exposed to VC (IARC, 1979, 1982).

All the epidemiological studies mentioned above on the carcinogenicity of VC in humans, were performed on people occupationally exposed.

The risk of cancer mortality was studied among residents (45 years of age or older) of three U.S.A. communities with VC–PVC plants. A higher death rate from central nervous system tumors was observed among males (Infante, 1976).

Just recently an increase in malignant melanomas has been found in a cohort of male workers producing VC and PVC (Heldaas et al., 1984).

Italian worker of
HE×200.

ker of VC-PVC
HE×200.

**TABLE 11**

**Tumors of different site and type, other than liver angiosarcoma, correlated with VC occupational exposure.**

| Site | Type | Comments | Country | References |
|---|---|---|---|---|
| Duodenum, heart, kidney, and other sites | Angiosarcoma | | USA | Thomas and Popper, 1975 |
| *Brain and central* nervous system | *Glioblastoma* multiforme | | USA | *Tabershaw and Gaffey, 1974;* Infante, 1976; Waxweiler et al., 1976; Cooper, 1981 |
| | | | Sweden | Byrén et al., 1976 |
| | | | FRG | Von Reinl et al., 1977 |
| Lung | Large-cell undifferentiated carcinoma | Atypical cells in sputum in workers exposed to VC (Maltoni et al., 1974 a; Maltoni and Lodi, 1981) | USA | Tabershaw and Gaffey, 1974; Waxweiler et al., 1976; Cooper, 1981 |
| | | | UK | Fox and Collier, 1976 |
| | | | Sweden | Byrén et al., 1976 |
| | | | FRG | Von Reinl et al., 1977 |
| Hemolympho-reticular system | Lymphomas and leukemias | | USA | Tabershaw and Gaffey, 1974 Waxweiler et al., 1976 |
| | | | FRG | Von Reinl et al., 1977 |
| Biliary tract | Carcinoma | | USA | Monson et al., 1974 |
| Pancreas | Carcinoma | | Sweden | Byrén et al., 1976 |
| All sites | All cancers | | USA | Nicholson et al., 1975 Ott et al., 1975; Cooper, 1981 |

*(b) Liver angiosarcomas, angiomas, and fibroangiomas.* The histopathological patterns and the natural history of these tumors are similar to the corresponding rat tumors (Fig. 54).

*(c) Angiosarcomas, angiomas, and fibroangiomas of other sites.* Angiosarcomas, angiomas and fibroangiomas are similar to the corresponding tumors observed in rats. They have been found in a variety of organs and tissues, apart from liver: mainly in subcutaneous tissue and peritoneum.

*(d) Hepatomas.* They are usually low deviation tumors, of the type usually seen in untreated mice of many different strains.

*(e) Forestomach papillomas and acanthomas.* The histopathological pictures of these tumors are similar to the ones found in rats.

*(f) Lung adenomas.* These tumors are usually multiple, superficial or intraparenchymal, with a nodular shape. They present a tubular papillary or solid pattern with or without cellular atypias: the atypias may be so pronounced to suggest an early adenocarcinomatous transformation (Fig. 55-58).

*(g) Cutaneous epithelial tumors.* They are benign and malignant in nature, namely: papillomas, acanthomas, and squamous cell carcinomas.

*C) Tumors in hamsters*

*(a) Liver angiosarcomas, angiomas, and fibroangiomas.* They are similar to the corresponding tumors observed in rats and mice, with a predominance of the papillary pattern (Fig 59).

*(b) Angiomas and fibroangiomas of other sites.* Histopathologically they are similar to the corresponding tumors observed in rats and mice, and have been found in subcutaneous tissue and spleen.

*(c) Forestomach papillomas and acanthomas.* The histopathological pictures of these tumors are similar to the ones observed in rats and mice.

*(d) Cutaneous epithelial tumors.* These tumors are usually found in the middle of the flank. Histopathologically they are trichoepitheliomas (Fig. 60), and basal cellular carcinomas. Often, within the trichoepitheliomas, there are areas suggestive of carcinomatous transformation.

*(e) Melanomas.* They may be located in different sites of the skin, and are strongly and poorly pigmented.

*(f) Acoustic duct epithelial tumors.* Trichoepitheliomas, acanthomas, basal cellular, and squamous cellular carcinomas have been observed.

*(g) Leukemias.* The leukemias are mainly found in the abdominal organs. Histopathologically they are of the lymphoimmunocytic type. The gross and microscopic pictures are very similar in all observed cases.



## V. CONCLUSIONS

VC dependent tumors were identified on the basis of one or more of the following parameters: 1) sharply enhanced incidence; 2) rare or exceptional occurrence in the colony of the animals used; 3) dose–response relationship; 4) association of precursor lesions.

From the results of the experiments of the BT project the following conclusions can be drawn:

1) VC causes tumors in all three animal species tested (Table 20);
2) VC is a multipotential carcinogen since it causes tumors of different types, in different sites (Table 20);
3) some types of tumors are observed in all three animal species studied (i.e. liver angiosarcoma), some types only in two (i.e. malignant mammary tumors, angiosarcoms of other sites, and lung tumors), and some types only in one (i.e. Zymbal gland carcinomas, nephroblastomas and neuroblastomas);
4) the degree of evidence of the correlation between VC treatment and the tumors considered as VC–dependent varies from tumor to tumor;
5) VC shows carcinogenic effects when administered by inhalation, by ingestion, and via placenta, and, possibly, by intraperitoneal and subcutaneous injection;
6) through inhalation and ingestion experiments there is a clear–cut dose–response relationship;
7) VC shows definite carcinogenic effects down to 5 ppm (4 hours/daily, 5 days weekly, for 52 weeks) by inhalation, and 0.3 mg/kg b.w. (once daily, 4–5 days weekly, for 52–59 weeks) by ingestion;
8) the duration of treatment greatly affects the carcinogenicity of VC: keeping all of the other experimental factors constant, the longer the treatment is, the higher the neoplastic response. This effect is more evident for some tumors than for others;
9) the neoplastic response, in qualitative and quantitative terms, is greatly affected by the species, strain, and sex of the animals studied;
10) newborn animals (rats) have been shown to be extremely more responsive to VC, with respect to the development of hepatocellular carcinomas and liver angiosarcomas;
11) perinatal (prenatal and neonatal) exposure is the crucial factor involved in enhancing the neoplastic response of the liver (development of liver angiosarcomas and of hepatocellular carcinomas) and of the lungs (development of lung adenomas), in rats;
12) the target organs (liver, brain, lung, hemolymphoreticular tissues) of VC carcinogenicity in exposed workers, and the "marker" tumor (liver angiosarcoma), were predicted by the results of the experiments of the BT project.

# Part 3
# THE HISTORY OF VC CARCINOGENICITY

After more than 40 years since the beginning of its industrial production, nothing was known about the carcinogenic risks of VC.

Specific studies on VC carcinogenesis began around the start of the 70's.

The most important steps towards the knowledge of VC carcinogenicity, including specific oncological research and toxicological pertinent contributions, are listed in Table 61.

## TABLE 61
## THE HISTORY OF VINYL CHLORIDE CARCINOGENICITY.

| | |
|---|---|
| 1) 1961: | VC was found to produce liver enlargement and microscopic hepatic degenerative changes in mice (Torkelson et al., 1961). |
| 2) 1970: | Zymbal gland carcinomas were reported in rats exposed to 30,000 ppm of VC, by inhalation (Viola et al., 1971). |
| 3) 1970: | An increase in atypias in respiratory cells was observed among workers heavily exposed to VC (Maltoni et al., 1974 a). |
| 4) 1971 (July): | A vast project of long-term carcinogenicity bioassays on VC was started in Bentivoglio, near Bologna, Italy (BT project). |
| 5) 1972 (August): | Zymbal gland carcinomas, nephroblastomas and liver angiosarcomas were observed in rats exposed to VC by inhalation (Maltoni, BT project). |
| 6) 1973 (April): | The first data of the BT project were released to the Scientific Community: the oncogenic effect was observed up to 250 ppm (Maltoni, 1974 a, b). |
| 7) 1973: | Splenomegalic liver disease was found among polyvinyl chloride production workers (Marsteller et al., 1973). |
| 8) 1973 (December): | For the first time a case of liver angiosarcoma in a worker of polyvinyl chloride production was correlated to VC exposure (Creech and Johnson, 1974). |
| 9) 1974 (February): | On the basis of the BT project data indicating a carcinogenic effect at 250 ppm, OSHA proposed a TLV of 50 ppm (Maltoni, 1974 b). |
| 10) 1974 (February): | The BT project data showed that VC is a multipotential carcinogen, producing a variety of tumors, in different animal species (Maltoni, 1974 b, c). |
| 11) 1974: | The BT project data indicated a carcinogenic effect at 50 ppm. OSHA proposed new stricter rules (Maltoni, 1974 c; Maltoni and Lefemine, 1975). |
| 12) 1974: | Early epidemiological observations (paralleling the experimental information) indicated an increase in tumors other than liver angiosarcomas (of brain, lung, hepatocytes, haemolymphoreticular tissues) among workers of VC-PVC industries (Wagoner, 1974) |
| 13) 1974-75: | BT project data showed that VC had carcinogenic effects in rats also when given by ingestion (Maltoni et al., 1975 a, b). |
| 14) 1976: | In rats of the BT project exposed to VC by inhalation, angiosarcomas were observed down to the level of 25 ppm, and Zymbal gland carcinomas down to the level of 10 ppm (Maltoni, 1977 a). |
| 15) 1974, 1983: | Two cases of liver angiosarcoma were observed among PVC extruders in a North-Italian plastic bag factory (Maltoni et al., 1983). This finding points out that also workers manufacturing and handling PVC must be considered at risk (Maltoni et al., 1983). |

# Part 4
## CONCLUSIVE COMMENTS

The experimental BT project and the entire body of research (experimental, epidemiological, pathological, clinical, environmental) which followed on VC carcinogenicity have brought forth important specific information, progress, decisions and facts in the field of VC carcinogenesis and of occupational and environmental carcinogenesis in general.

1) The studies on VC carcinogenicity have led to the control of VC exposure, and hopefully the avoidance of additional cases of VC related diseases.
2) They have promoted carcinogenicity studies of numerous chemically correlated compounds, to assess their potential risk.
3) They have stimulated national and international regulations and acts for the control of environmental toxic agents, encompassing the bills requiring pre–production testings.
4) They have constituted a model for research protocols in the field of studies on environmental and occupational carcinogenesis, including a large part of the ones enclosed in the Good Laboratory Practice Acts, for long–term carcinogenicity bioassays.
5) They demonstrated that experimental findings can be extrapolated to human pathology.
6) They have contributed to the basic knowledge of factors and mechanisms in cancerogenesis.
7) Human pathology has been enriched by the description of particular entities produced by VC exposure (first of all: liver angiosarcoma).
8) Finally, VC carcinogenicity has brought forth an important lesson: the studies in the field of environmental and occupational carcinogenesis, particularly in industrialized countries, must no longer be considered a retroguard ancillary research, but rather they must represent an important component of the decision–making processes which regulate the developmental trends of society.